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Enzymatic glucose biosensor based on manganese dioxide nanoparticles decorated on graphene nanoribbons

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Abstract A disposable glucose biosensor was prepared using nanoparticles of MnO_2 decorated on graphene nanoribbons by surface modification with drop coating with the GOx and Nafion®. Tested material was synthesized and characterized using several techniques. The biosensor could be operated under physiological conditions (0.1M phosphate buffer, pH 7.4) and exhibited good reproducibility and stability. The linear range for the amperometric response of the biosensor at operating potential of +0.50 (versus Ag/AgCl) was from 0.1 to 1.4 mmol/l, with a detection limit of 0.05 mmol/l and high sensitivity of 56.32 μA/mmol cm^2. Developed method was tested toward glucose quantification in real samples with satisfactory accuracy and precision.

Keywords: Glucose biosensor; Graphene nanoribbons; Manganese dioxide; Screen printed electrodes

Introduction

Determination of glucose is very important in numerous fields – clinical, biological and chemical as well as in food industry [1]. Many papers can be found in the literature that describes method for glucose analysis such as spectroscopy, chromatography and electrochemical methods. Electrochemical methods had been the most explored methods, considering their low cost, easy manipulation and good analytical performances [2]. The first developed amperometric enzyme electrode, based on glucose oxidase, was invented back in 1962 [3]. Since then until now, interest in the development these biosensors has not decreased. The main goal of many authors is development of new glucose biosensor with lower detection limit, wider linear scope and greater selectivity.

Glucose biosensors can be divided on enzymatic and non-enzymatic. Although, significant efforts have been invested in development of non-enzymatic sensors, satisfactory sensitivity, stability and reproducibility has not been achieved yet [4]. The biosensors based on enzyme glucose-oxidase are the most researched due to their high selectivity. The main problem of enzyme based biosensor is the influence of various factors (temperature, pH values, toxic elements) on the enzyme activity [5]. Attempts to resolved these problem were made by using nanomaterials such as metal nanoparticles [6–8], metal nanowires [9,10], metal oxides nanoparticles [11], carbon nanotubes [12,13]. These materials improve analytical performances of proposed biosensor due to their conductivity, high surface to volume ratio and good biocompatibility [14]. In the recent years, many researches developed novel analytical devices

based on nanostructured metal oxides that are cost-effective, highly sensitive due to the large surface-to-volume ratio of the nanostructure, and show excellent selectivity when coupled to biorecognition molecules [15]. One of these metal oxides, often used for fabrication of different kinds of biosensors is MnO_2 [16–20].

Graphene (GR) is a planar sheet of carbon atoms bonded by sp2 bonds. This configuration provides the material with extraordinary properties such as large surface area, high mechanical strength, high electrical conductivity, high elasticity and thermal conductivity [21,22]. Graphene nanoribbons (GNR) present narrow stripes of graphene or single-layer graphite and its electronic properties combine the properties and structure of carbon nanotubes (CNT) and graphene nanosheets (GNS) [23]. However, in comparison with CNT and GNS whose application in biosensor development has been widely explored, there are only few reports on usage of GNR in biosensing [23–25].

This paper describes a disposable electrochemical biosensor for glucose monitoring. According to our best knowledge, for the first-time graphene nanoribbons modified with nanoparticles of manganese dioxide were used to develop glucose biosensor. We have used the mentioned material with immobilized glucose oxidase upon screen printed carbon electrode for determination of bonded glucose in honey samples.

Experimental

Reagents and chemicals

All chemicals used in this paper were of analytical grade and used as supplied, without any further purification. Graphene nanoribbons (length 2-15 μm, width 40-250 nm) and glucose oxidase from *Aspergillus niger* (lyophilized powder, 100-250 units/mg solid) were supplied by Sigma Aldrich. For preparation of phosphate buffer (0.1M) with different pH values, corresponding amounts of sodium dihydrogen phosphate dihydrat and sodium hydrogen phosphate dihydrat were dissolved in ultra-pure water. D (+) glucose monohydrate, fructose, lactose, ascorbic acid, hydrogen peroxide (30%), and DMF (99.8%) were supplied by Merck. Solution of glucose (0.1M) was prepared by dissolving appropriate amount of glucose in ultra-pure water and left for 24 hours in order to mutarotate at room temperature.

Instrumentation

Cyclic voltammetry (CV) measurements and hydrodynamic chronoamperometry (HChA) measurements were performed using a potentiostat/galvanostatAutolab PGSTAT 302N (Metrohm Autolab B.V., The Netherlands) controlled by software Nova 2.0. All electrochemical experiments were done in conventional three electrode glass cell (total volume of 25 ml). An Ag/AgCl electrode (3 M KCl) was used as reference electrode and Pt wire as counter electrode. The working electrode was screen-printed biosensor electrode. Each potential reported in this paper is given against Ag/AgCl/3 M KCl electrode at a laboratory temperature of 25±1º C. For pH measurements pH meter model Orion 1230 equipped with combined glass electrode model Orion 9165BNWP (USA) was used.

X-ray powder-diffraction (XRD) analysis was performed on a high-resolution SmartLab® X-ray diffractometer (Rigaku, Japan) using Cu Kα radiation (λ = 0.1542 nm). The data were collected in the 2θ range from 10° to 75° in steps of 0.05° and with exposition of 2 s per step with divergent slit of 0.25 mm, operated at 40 kV and 30 mA. FT-IR spectra were recorded in mid IR range (4000–500 cm^{-1}) using a Nicolet iS50 FT-IR, Thermo Fisher Scientific spectrophotometer equipped by Smart iTR attenuated total reflectance (ATR) sampling accessory by placing powder samples on diamond plate and fixating them with pressure tower.

Microstructure and morphology of synthesized samples were investigated using a field emission-scanning electron microscope FE-SEM MIRA3 (Tescan, Czech Republic) coupled with an EDS analyser (Oxford, UK). The samples were prepared by dispersing the powder in acetone. Diluted dispersions were then dropped on a carbon-coated copper grid and left to dry for FE-SEM observations.

Procedures

Preparation of SPCE

The screen printed carbon electrodes were produced from carbon ink (No. C50905DI, Gwent, Pontypool, UK) and laser pre-etched ceramic supports (No. CLS 641000396R, Coors Ceramics GmbH, Chattanooga, TN, USA). Thick layers of carbon ink were formed by brushing the ink through an etched stencil (thickness 100 mm, electrode printing area 105 mm^2) with the aid of a

screen printing device (SP-200, MPM, Franklin, MA,USA) onto the substrates. The resulting plates were dried overnight at room temperature.

Synthesis of MnO_2-GNR composites

For the synthesis of nanoparticles of MnO_2 dispersed on GNR some modified procedure, than that reported in papers [26], was used in order to achieve the most optimal coverage of graphene nanoribbons with nanoparticles of manganese dioxide. Firstly, 5 mg of GNR was dissolved in 5 ml of water and ultra-sonicated for 1 hour. Manganese (II) nitrate tetrahydrat (21.8 mg) was dissolved in 45 ml of water. These two solutions were mixed and ultra-sonicated for 2 h, forming a uniform brown dispersion. Solution of $KMnO_4$ was obtained by dissolving 0.0457 mg of $KMnO_4$ in 50 ml of water. This solution was added into $Mn(NO_3)_2$/GNR dispersion under vigorous stirring. After magnetic stirring for 6 hours, the obtained composite of MnO_2 and GNR was centrifuged, washed three times with ultra-pure water and at the end three times with ethanol. Composite was dried at room temperature overnight. At the same way, only without adding GNR, nanoparticles of MnO_2 were prepared. After that, samples were weight and dissolved in appropriate amount of DMF in order to obtain final concentration of composite (1 mg/ml).

Preparation of working electrode (MnO_2-GNR/SPCE) and biosensor

The preparation of modified electrode was done as follows: 5 µl of MnO_2/GNR was applied at SPCE and let to dry overnight. Also, in order to compare electrochemical properties of obtained materials, one electrode was prepared by applying only MnO_2 nanoparticles on SPCE surface. For preparation of biosensor, solution of glucose oxidase was prepared by dissolving 0.1 gram of enzyme in 10 ml PBS buffer solution (pH 7.4). This solution was kept in refrigerator until use. On obtained electrode, MnO_2-GNR/SPCE, 5 µL of glucose oxidase (GOx) solution were added, and dried at 4°C overnight. After this period, 2.5 µL of Nafion (0.5% Nafion in ethanol) was dispread at electrode surface and allowed to dry at 4°C.

Results and discussion

Characterization of working electrode

The crystalline structure of the products was analysed by XRD. The XRD patterns for GNR, MnO_2 and MnO_2/GNR nanocomposites are shown in Figure 1a. The pattern of GNR (black line) shows a sharp diffraction peak at about $2\theta = 26.1°$ corresponding to a d-spacing (interlayer spacing) of 3.4 Å, which is consistent with the results for graphene and CNTs [27]. The sharp XRD peak indicate that sample is highly crystalline, with the crystal size of GNRs, calculated using the Scherrer equation considering the (002) plane, is 26.6 nm. Two small peaks at 2θ of 42.8° and 54.1° can be indexed to the (101) and (004) planes of graphite. The XRD pattern of MnO_2 is shown in red line of Figure 1. The diffraction peaks at 2θ values of 36.7° and 66.0° were observed. These peaks could be ascribed to the (211) and (002) crystal planes of α-MnO_2 [28]. However, these peaks are broadened, which indicates the poorer crystallinity of MnO_2. The pattern of MnO_2/GNR nanocomposite (blue line) showed all peaks found in previous samples, which confirmed incorporation of MnO_2 into GNR structures.

FT-IR spectra of GNRs, MnO_2 and MnO_2/GNR composite are shown in Figure 1b. The IR spectrum of GNRs (black line) was fairly different from that of GO found in literature [29,30], with almost all characteristic peaks reduced. There is only small absorption at 1065 cm^{-1} due to the C–O (alkoxy) vibration. Spectrum of MnO_2 (red line) shows sharp stretching vibrations that appeared in the range 2965–2836 cm^{-1} that are due to the stretching vibration of C–H bonds. The wavenumber region 1390–1750 cm^{-1} is the stretching vibration of the carbonyl group. There is slight sign of absorption of the Mn–O lattice vibrations at about 508 cm^{-1} which confirms formation of MnO_2 [31].

<<< Preferred position for Figure 1>>>

The morphology of as prepared materials was further observed using FE-SEM (as seen in Figure 2). Figure 2a shows the morphology of GNRs, with long curved rod-like structures of GNR. MnO_2 nanospheres are aggregated in larger agglomerates with average particle size of ~300 nm, as shown in Figure 2b. MnO_2 structures are closely and homogeneously grown on the GNR support (Figure 2c) which can be seen from EDS mapping also (Figure S1).

<<< Preferred position for Figure 2>>>

Figure 3. presents the voltammetric characterization of SPCE, GNR/SPCE, MnO_2/SPCE and MnO_2-GNR/SPCE by cyclic voltammetry performed in 0.1 M phosphate buffer (pH 7.4) in presence of 5 mM $K_3[Fe(CN)_6]$ /$K_4[Fe(CN)_6]$ with a scan rate 50 mV/s in a potential window from -1.0 to +1.0 V. The best response was achieved with MnO_2-GNR/SPCE electrode, regarding peak shape and peak current. Also, with GNR/SPCE electrode, an expanded peak was obtained, but was the same order of magnitude as in the case of MnO_2-GNR/SPCE electrode. As for bare SPCE and MnO_2/SPCE electrode, there were no distinctly signals noticed. As can be concluded, GNR significantly improves characteristic of electrodes while nanoparticles of manganese dioxide can significantly contribute to the reduction of interference [32,33].

<<< Preferred position for Figure 3>>>

Influence of pH value of supporting electrolyte at analyte signal was investigated. Cyclic voltammograms were recorder for 0.1 M buffer solution in the pH range from 6 to 8, containing 25 mM H_2O_2. As shown at Figure S2, there were no significant differences between obtained voltammograms, and for all further examination we have chosen pH values of 7.4, most similar to biological pH.

Analytical procedure for the determination of H_2O_2 and glucose

Before all chronoamperometric experiments the electrodes were electrochemically activated and conditioned by potential cycling from -1.0 to 1.0 V for four cycles with a scan rate of 100 mV/s in 0.1 M phosphate buffer supporting electrolyte (pH 7.4) in order to achieve stable chronoamperometric baseline yielding higher repeatability of the method [34]. First, we have investigated the influence of applied potential on amperometric response of 25 mM H_2O_2 in 0.1 M PBS solution pH 7.4. As can be seen on Figure 4, current response of analyte raises with applied potential. As optimal potential we have chosen 0.5 V, having in mind that with higher potential background current is much higher as well and also there are numerous compounds that can interfere with the determination of glucose at higher potentials [35,36].

<<< Preferred position for Figure 4>>>

The chronoamperometric responses of hydrogen peroxide, at selected working potentials, show staircase-like signals with all examined electrodes (Fig 5). The best analytical parameters were obtained for MnO_2-GNR/SPCE and for MnO_2/SPCE electrode. In the case of bare SPCE and GNR/SPCE electrodes, signals stability and repeatability were very poor. As for SPCE electrode modified with MnO_2, results were much better, but still current response was lower than in the case MnO_2-GNR/SPCE. Also, signal stability and response permanency were the best in this case. Obviously, it can be concluded that synthesized composite strongly increased electrocatalytic performances toward hydrogen peroxide, probably due to synergetic effect of graphene nanoribbons and manganese dioxide, and that proposed approach can be promising for construction of sensitive and stable glucose biosensor.

<<< Preferred position for Figure 5>>>

Calibration curve obtained for hydrogen peroxide, in 0.1M PBS buffer (pH 7.4) at potential of 0.5 V is shown at Figure 6. Chronoamperometric current response shows linear dependence with increase of hydrogen peroxide concentrations in the range of 0.5-13 mM and this linearity can be expressed with following equation $I\ (\mu A) = 0.078\ c\ (\mu M) - 0.193$ with regression coefficient of $R^2 = 0.998$.

<<< Preferred position for Figure 6>>>

The characteristic parameters for quantification of hydrogen peroxide using all examined electrodes are listed in the Table 1. As can be seen best results, widest linear range, lowest limit of detection, limit of quantification and best linear regression coefficient, are achieved with composite material, confirming above mentioned statements that synergetic effect of MnO_2 and graphene nanoribbons are crucial to obtain best characteristics of proposed electrochemical biosensor.

Table 1. Selected analytical parameters for hydrodynamic chronoamperometric determination of H_2O_2 with an SPCE, GNR/SPCE, MnO_2/SPCE and MnO_2-GNR/SPCE in phosphate buffer (pH 7.4), operating potential +0.50V

	Analytical parameters			
Electrodes	Range, mmol/l	LOD, mmol/l	LOQ, mmol/l	r
SPCE	0.5-11	0.28	0.92	0.999

GNR	0.5-7	1.37	4.57	0.988
MnO_2	0.5-11	0.23	0.76	0.998
MnO_2-GNR/SPCE	0.5-13	0.22	0.72	0.999

Based on previously obtained results, proposed glucose biosensor was constructed from new, modified electrode (MnO_2-GNR/SPCE) by surface modification with drop coating with the GOx and Nafion®. Detailed preparation procedure is given in the Experimental section. Amperometric determination of glucose has been investigated and obtained amperogram is shown on Figure 7. Inset figure present calibration curve of response current to glucose concentration. Linear range for our method is from 0.1 to 1.4 mmol/l, with a correlation coefficient of 0.9982. This biosensor has good detection limit of 0.05 mmol/l and high sensitivity of 56.32 μA/mmol cm^2 and a short response time (within 10s). Comparing our results (Table 2) it can be noticed that proposed approach offer comparable or better characteristics (limit of detection, linear range, sensitivity) with some recently reported literature data.

Table 2. A comparison of the electrocatalytic performances of GOx/Naf/MnO_2-GNR/SPCE with other enzymatic glucose sensors

Electrode	Applied potential (V)	Range (mM)	LOD (mM)	Sensitivity (μA/mmol cm^2)	Reference
PET/VACNT-Al foil/PFLO/GO_x	-0.7	0.02-0.5	0.007	65.816	[37]
RGO-GO_x/GC	-0.44	0.21-27	Not given	1.85	[38]
GOx/Ag@MWCNT-IL-Fe_3O_4/MGCE	-0.51	0.006-2	0.00212	Not given	[39]
GOx-CS/AgNWs/GCE	-0.15	0.01-0.8	0.00283	Not given	[9]
MWCNT/GOx/Nafion	+0.4V	0.001-0.5	0.001	12.1	[13]
PPy-GOx/PPy-Cl	+0.7	0.5-24	0.027	3.5	[40]
AuNPs/BSA/Fe_3O_4/Pt	+0.4	0.25-7	0.003	115.3	[8]
GOx/Naf/MnO_2/GCE	+0.7	0.2-3.8	0.026	38.2	[41]
GOx-SiO_2/Lig/CPE	+0.6	0.5-9	0.145	0.78	[42]
GOx/Naf/MnO_2-GNR/SPCE	+0.5	0.1-1.4	0.050	56.32	Our work

Repeatability of the proposed approach was tested by measuring 0.5 mmol of glucose during five days using same the electrode. Reproducibility of the electrode construction pathway was tested with detection of 0.5 mmol of glucose with 4 independently prepared electrodes. Relative standard deviations obtained for these measurements of 3.5 and 4.7 %, respectively, clearly indicate that this approach offer stable, precise and accurate method for detection of glucose. Life time of the electrode was tested, too. It was considered that current differences higher of 10 % are leading to the conclusion that electrode life time is finished. During nonworking time electrode was stored at +4 °C in the refrigerator. Life time, obtained on this way, for our constructed electrode was 8 days. All mentioned facts indicate that developed sensor can be tested for application in glucose content detection in the real samples.

<<< Preferred position for Figure 7>>>

Interferences studies

Selectivity of the developed electrochemical procedure was tested with common species that can be found as accompanying molecules with glucose. Ascorbic acid (AA) and dopamine (Dop) were tested as interferences for the samples such as blood and blood serum, while lactose (Lac) and fructose (Fru) were tested as widely presented sugars in different samples. As can be seen, addition of ascorbic acid and dopamine cause current change indicating interfering effect of these molecules. On the other hand, after addition of 2.5 higher concentrations of fructose and lactose, the interference was not observed.

<<< Preferred position for Figure 8>>>

Real sample analysis

Practical application of the biosensor for the determination of glucose level in honey samples, was performed. For analysis of the samples three different meadow honey samples were used. Around 0.1 g of the samples was dissolved in water and transferred into 25 ml volumetric flask. Aliquot of 1.00 ml of the samples was added in 25 ml electrochemical cell with supporting electrolyte and analyzed. Recovery tests were done in order to investigate matrix effect. Results are listed in Table S1. Satisfactory agreement of our results with those obtained with standard

glucometer clearly indicate that this method offers precise and accurate methodology for estimation glucose concentrations.

Conclusions

In this work we have proposed electrochemical biosensor for the detection of glucose based on synthesis of composite material MnO_2/GNR. These studies shows that synergetic effect of manganese dioxide decorated of graphene nanoribbons increase characteristics of the electrode surface as well as final characteristics of the developed biosensors and that selection of appropriate material can play crucial role in obtained results. Herein proposed procedure offer satisfactory selectivity, sensitivity, accuracy and precision and as it is shown it can be successfully applied for application in real samples analysis.

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Figure caption.

Figure 1. a) XRD patterns of GNR, MnO_2 and MnO_2/GNR composite, b) FT-IR spectra of GNR, MnO_2 and MnO_2/GNR composite

Figure 2. FE-SEM micrograms of a) GNR; b) MnO_2 and c) MnO_2/GNR composite.

Figure 3. Cyclic voltammograms for 5 mM $K_3[Fe(CN)_6]$ /$K_4[Fe(CN)_6]$ in 0.1M phosphate buffer (pH 7.40) using SPCE, GNR/SPCE, MnO_2/SPCE and MnO_2-GNR/SPCE. Scan rate 50 mV/s

Figure 4. Effect of applied potential on the response current to 25 mM H_2O_2 at MnO_2-GNR/SPCE electrode in 0.1M phosphate buffer solution (pH 7.40)

Figure 5. Hydrodynamic chronoamperograms of H_2O_2 recorded with SPCE, GNR/SPCE, MnO_2/SPCE and MnO_2-GNR/SPCE in phosphate buffer (pH 7.4), operating potential +0.50V.

Figure 6. A) Chronoamperograms of H_2O_2 recorded with MnO_2-GNR/SPCE in phosphate buffer (pH 7.4), operating potential +0.50V B) Calibration curve for chronoamperometric response of MnO_2-GNR/SPCE electrode to addition of aliquots 0.1 and 0.2 mM of H_2O_2

Figure 7. A) The amperometric response of GOx/Naf/MnO_2-GNR/SPCE at +0.5 V upon successive addition of glucose (0.1 mM; 0.2 mM; 0.3 mM; 0.4 mM; 0.6 mM; 0.8 mM; 1.0 mM; 1.2 mM; 1.4 mM) in 0.1 M PBS buffer (pH 7.4) B) Calibration curve for chronoamperometric response of MnO_2-GNR/SPCE electrode to addition of glucose

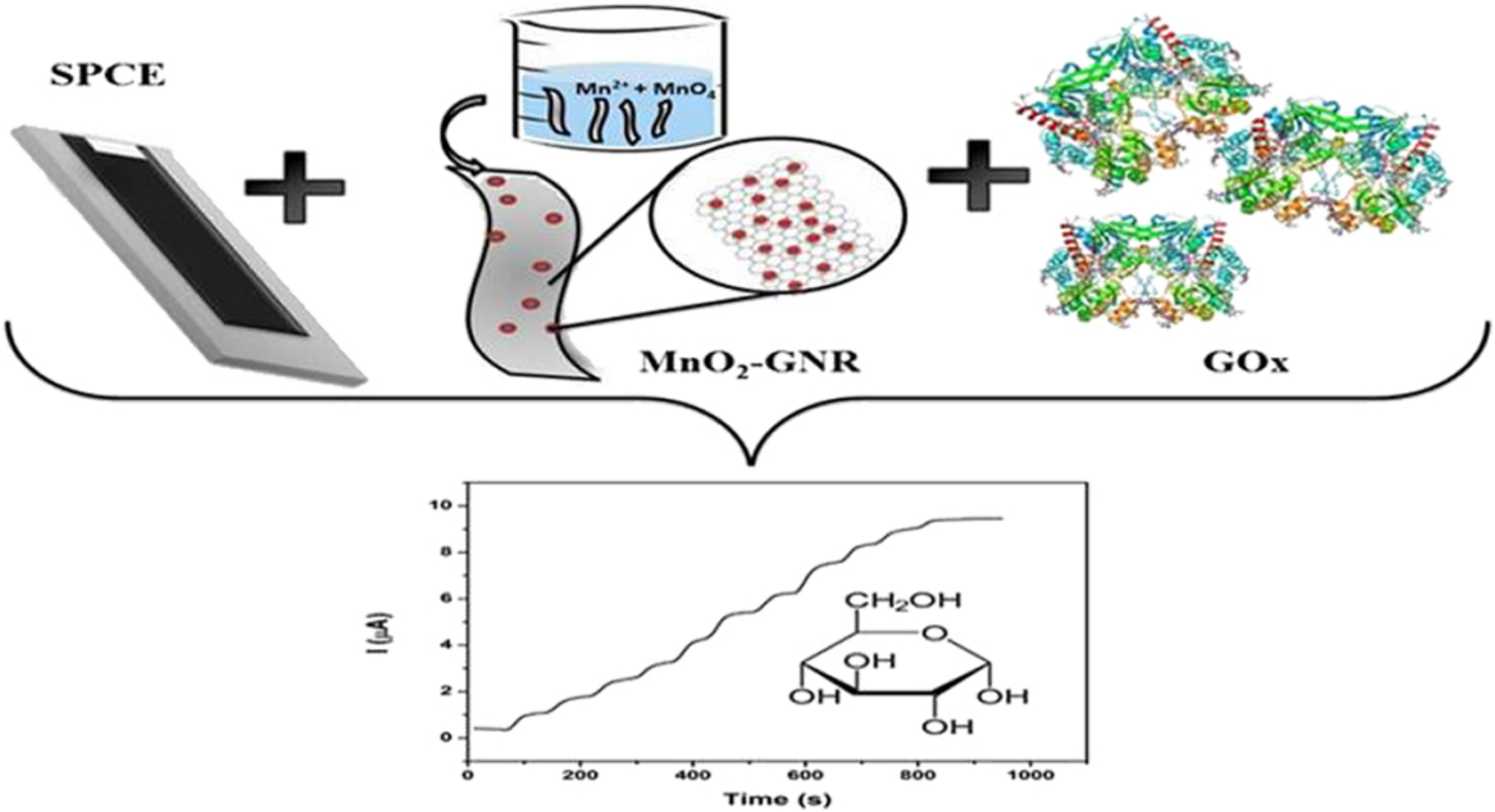
Figure 8. Amperometric response of the GOx/Naf/MnO2-GNR/SPCE electrode to glucose (0.4 mmol), lactose, fructose (1 mmol), ascorbic acid (0.2 mmol) and dopamine (0.2 mmol) in 0.1M phosphate buffer solution (pH 7.4)

Highlights

New enzymatic biosensor for the determination of glucose is proposed.

Development of a disposable biosensor based on graphene nanoribbons supported with MnO_2 nanoparticles.

Satisfactory selectivity, sensitivity and precision of proposed method are obtained.



Graphics Abstract

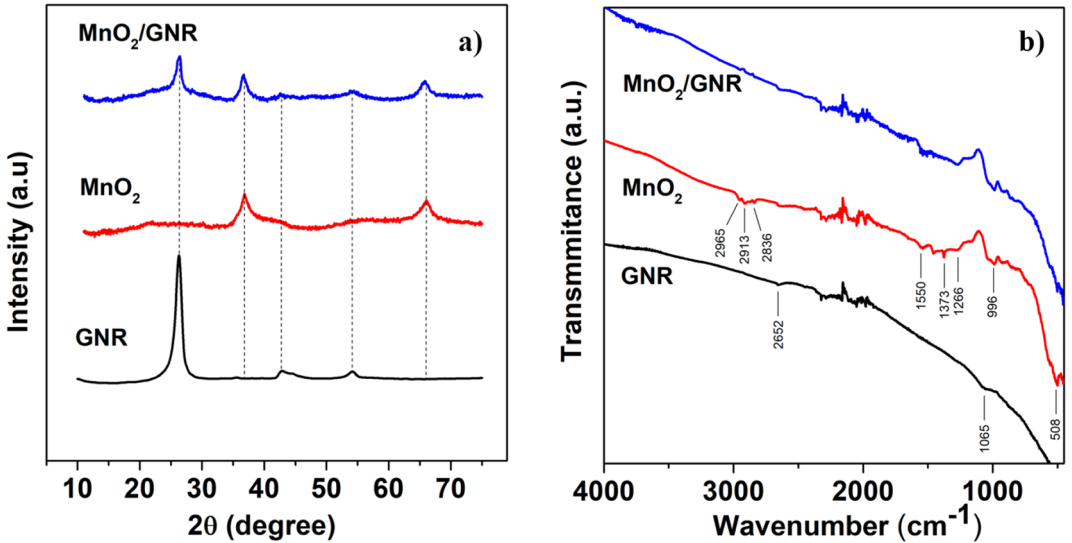

a)
MnO2/GNR
MnO2
GNR
Intensity (a.u)
10
20
30
40
50
60
70
2θ (degree)
b)
MnO2/GNR
MnO2
GNR
Transmmitance (a.u.)
2965
2913
2836
2652
1550
1373
1266
996
1065
508
4000
3000
2000
1000
Wavenumber (cm-1)

Figure 1

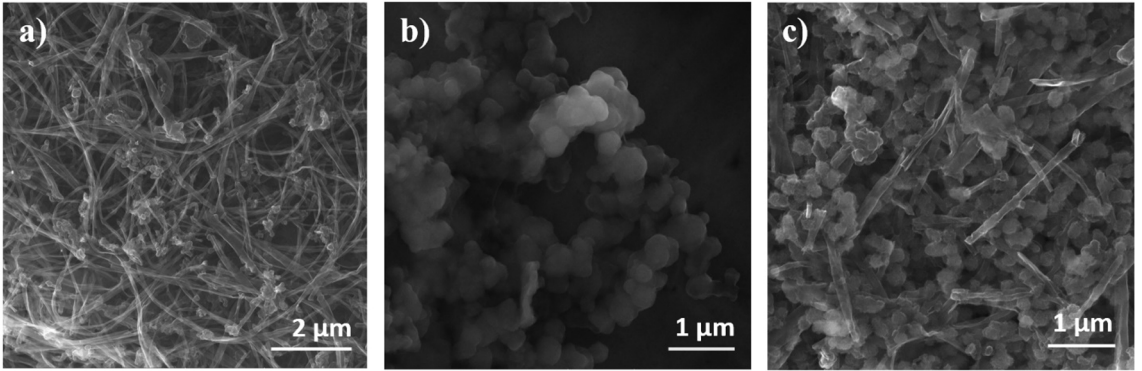


Figure 2

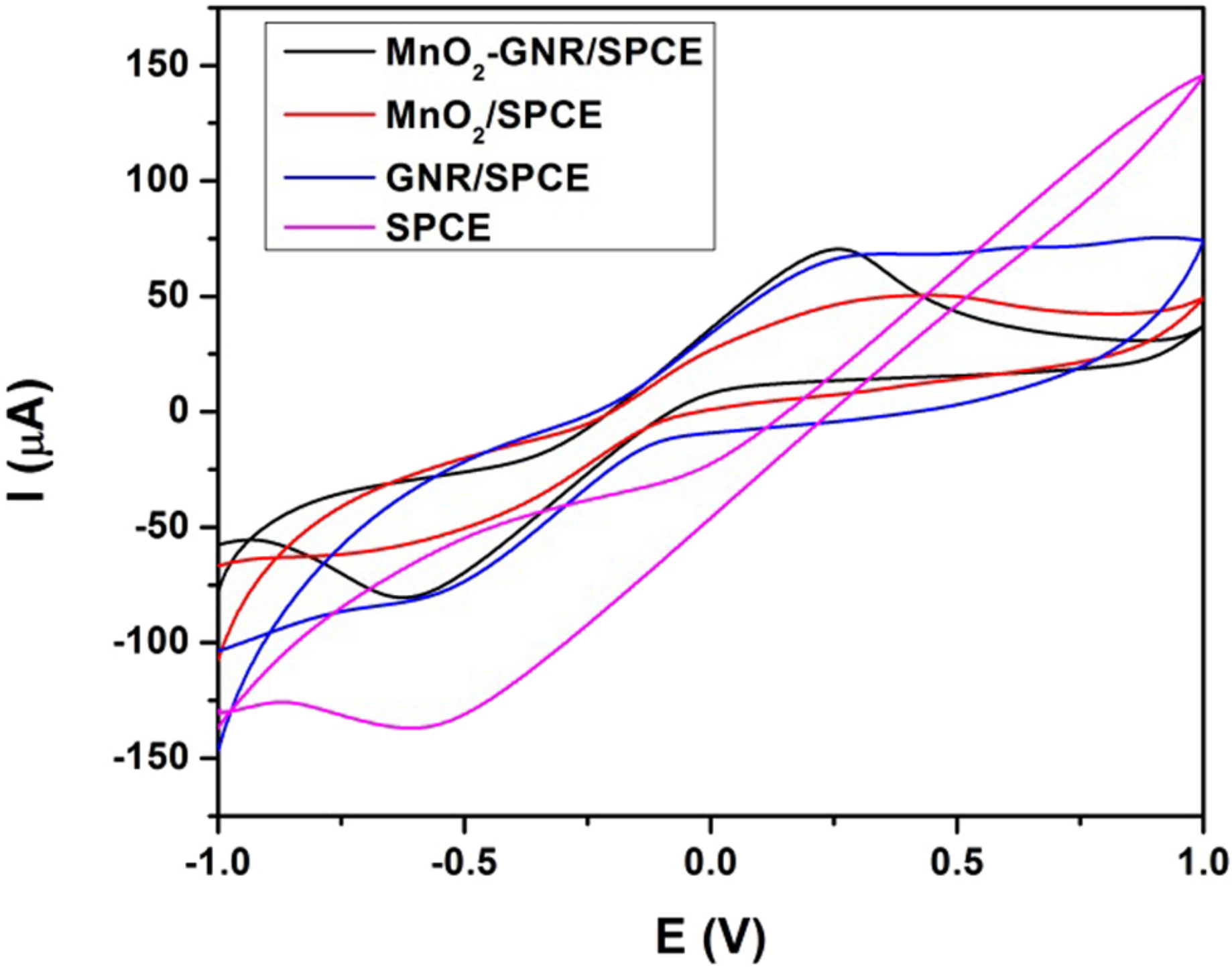

MnO2-GNR/SPCE
MnO2/SPCE
GNR/SPCE
SPCE
150
100
50
0
-50
-100
-150
I (μA)
-1.0
-0.5
0.0
0.5
1.0
E (V)

Figure 3

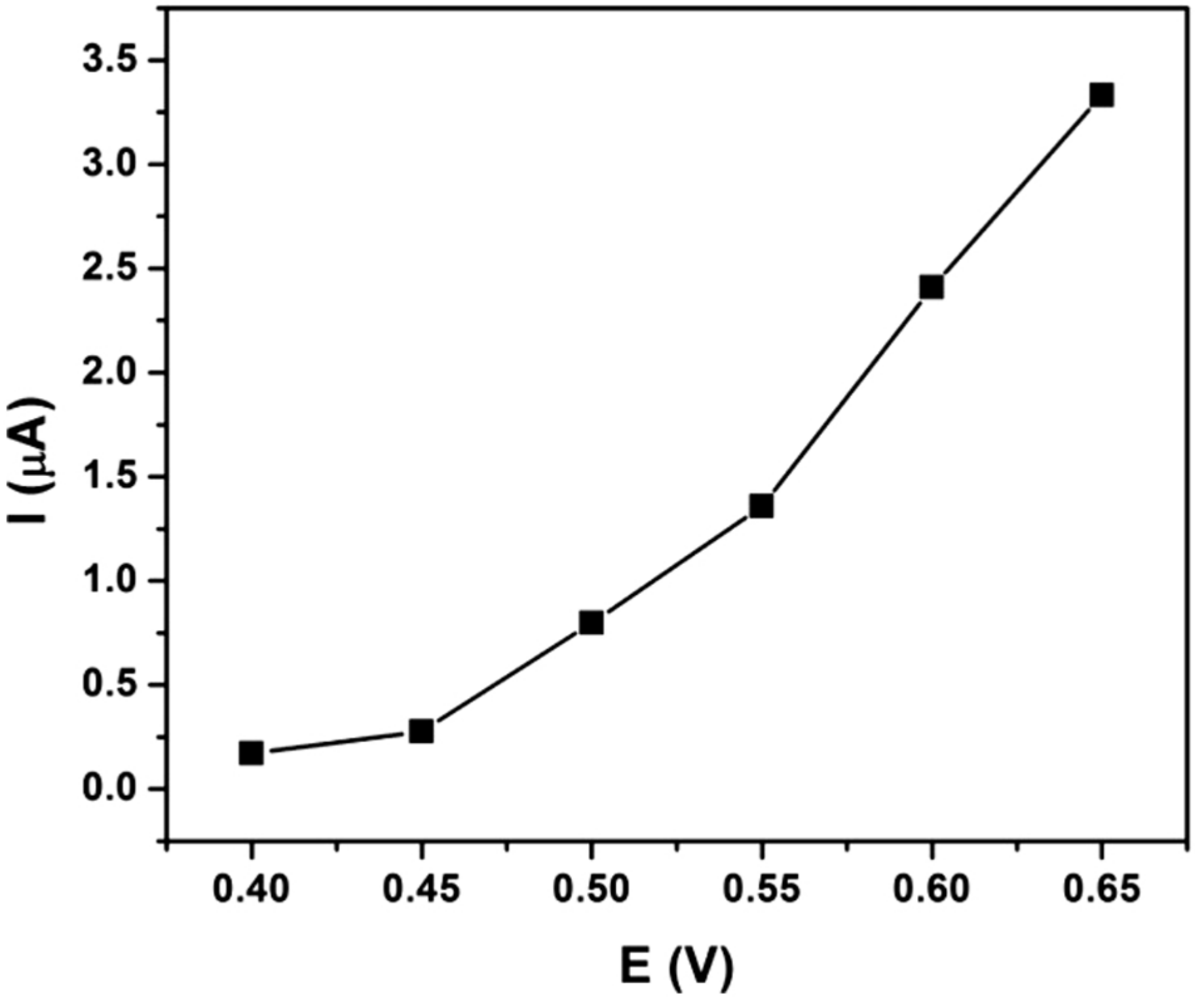


Figure 4

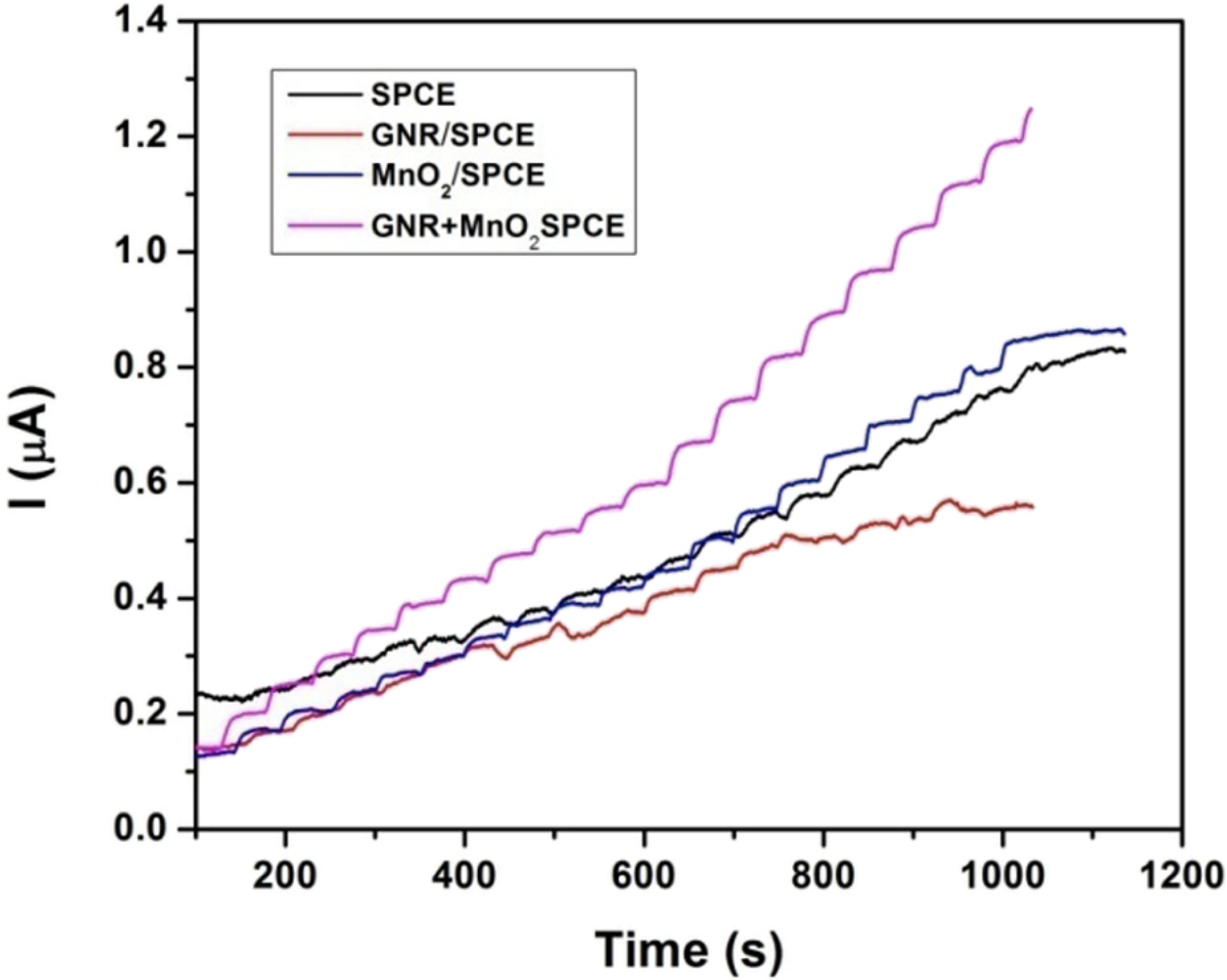

SPCE
GNR/SPCE
MnO2/SPCE
GNR+MnO2SPCE
I (μA)
Time (s)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
200
400
600
800
1000
1200

Figure 5

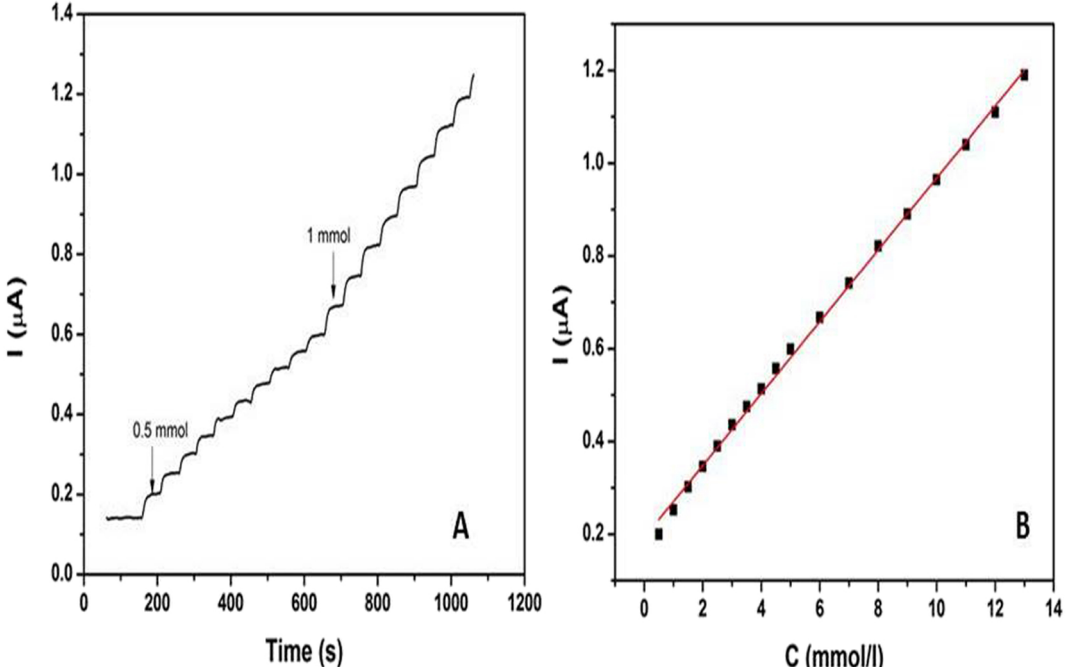


Figure 6

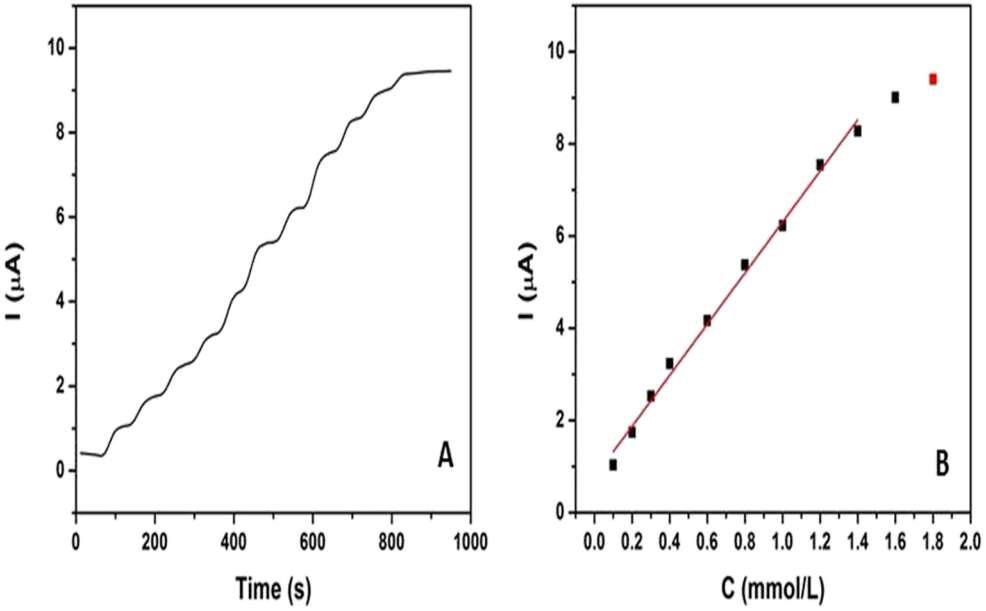


Figure 7

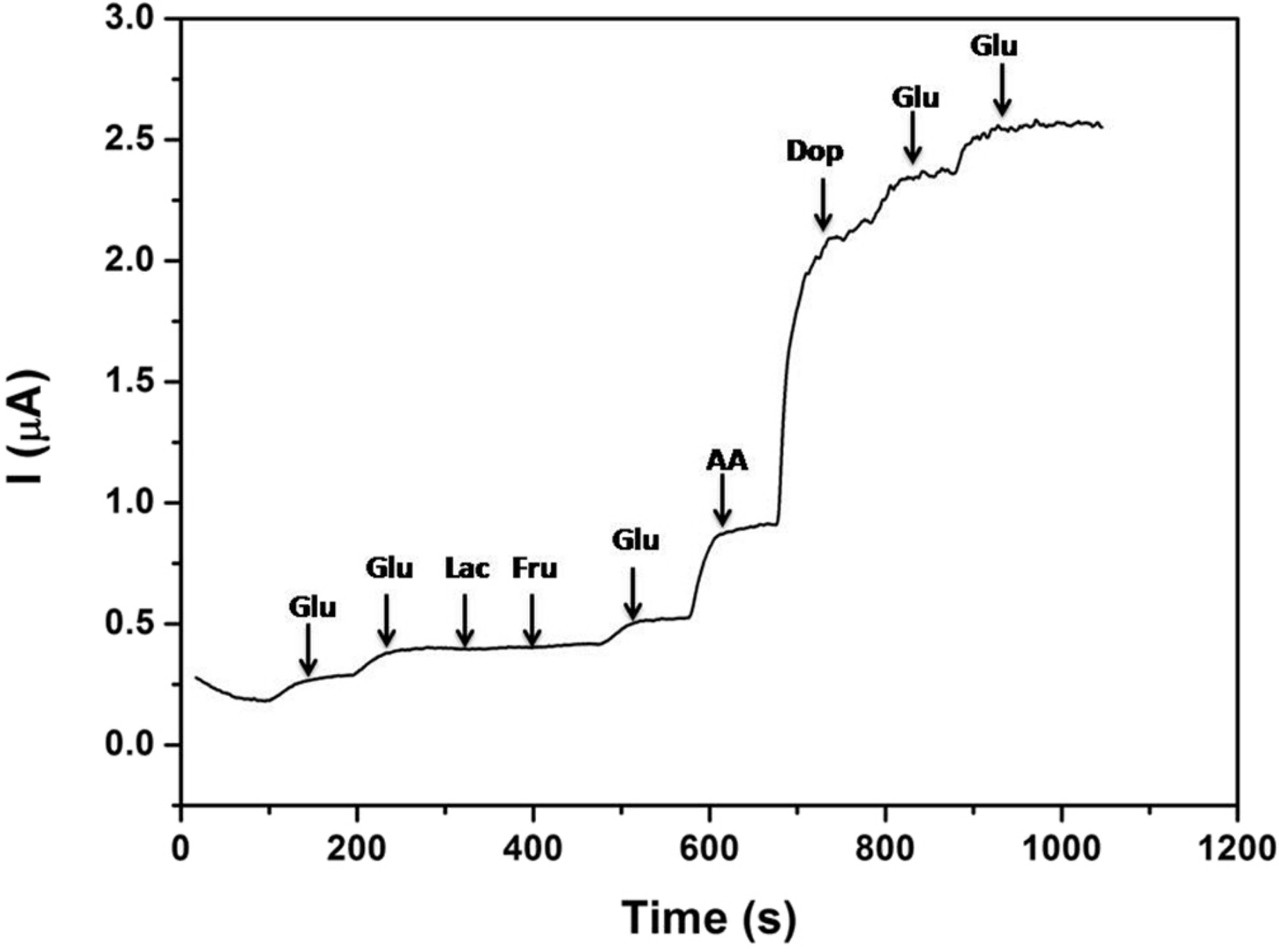

3.0
2.5
2.0
1.5
1.0
0.5
0.0
I (μA)
0
200
400
600
800
1000
1200
Time (s)
Glu
Glu
Lac
Fru
Glu
AA
Dop
Glu
Glu

Figure 8