

Paper-Based Biosensor for Glucose and Paracetamol Sensing using Chitosan Graphene Oxide Modified Electrode -

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Submission date: 05-Nov-2020 08:06AM (UTC+0700)

Submission ID: 1436432675

File name: osan_Graphene_Oxide_Modified_Electrode_-_Hilya_Nur_Imtihani.docx (252.74K)

Word count: 3562

Character count: 21614

RESEARCH ARTICLE

Paper-Based Biosensor for Glucose and Paracetamol Sensing using Chitosan/ Graphene Oxide Modified Electrode

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Received: 19th March, 2020; Revised: 26th April, 2020; Accepted: 22nd May, 2020; Available Online: 25th June, 2020

ABSTRACT

This work presents the development of chitosan/ graphene oxide modified paper-based electrode for electrochemical sensing glucose and paracetamol. Preparation of the modified paper-based electrode was described, and several characterizations using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) were carried out to evaluate the physical, electronic, and microstructural properties of chitosan/ graphene oxide layer. The electrochemical performance of the modified electrode was evaluated preliminary using impedance spectroscopy, and the electrochemical detection of both glucose and paracetamol in simulated urine was assessed using cyclic voltammetry (CV). Impedance data reveals that the electron transfer mechanism at the electrode/ analyte solution interface occurs faster with increasing concentration of the analyte. CV-based measurements exhibit a linear response for analyte concentration ranging between 2.5 and 100 μ M. In addition, irrespective of the analyte, the sensitivity increases with an additional fraction of graphene oxide in the modified paper-based electrode. The highest sensitivity for glucose sensing is 4.4 mA.mM⁻¹, while the highest sensitivity for paracetamol sensing is 16 mA.mM⁻¹, by using electrode coated with chitosan/ graphene oxide (5%, %w) composites.

Keywords: Chitosan, Environmentally friendly, Graphene oxide, Paper-based biosensor.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.2.18

How to cite this article: Roekmono, Hadi H, Imtihani HN, Wahyuono RA. Paper-based biosensor for glucose and paracetamol sensing using chitosan/graphene oxide modified electrode. International Journal of Drug Delivery Technology. 2020;10(2):295-300.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Microfluidic paper-based analytical devices (μ PADs) with sensors used to detect the target compounds develop rapidly for affordable clinical diagnostic technologies.^{1,2} Among various sensing techniques, electrochemical technique, using either voltammetry or electrochemical impedance spectroscopy (EIS), is intensively exploited as this technique enables fast response, high sensitivity, and selectivity, and enables the development of detection devices portable.³⁻⁵ Li and co-workers,⁶ for example, fabricated an electrochemical sensor on ZnO functionalized paper-based substrates, and have successfully performed CV to detect glucose. Further functionalization of the paper-based substrate using other functional nanomaterials, e.g., carbon nanotubes, metal oxide semiconductor, and magnetic particles, becomes more attractive and prospective to develop electrochemical biosensor.^{7,8}

With regard to the quest for developing high performance μ PADs systematic studies to optimize the design of microfluidic paper channel, as well as, the sensing properties

have been reported^{7,8}: a microfluidic paper decorated with ZnO nanoparticles⁹ and ZnO nanoflower¹⁰ enables separation of blood plasma and detection of bio compounds targeted in blood plasma using CV and EIS. Besides, electrochemical detection has been improved by compositing the nanostructured ZnO with conductive multi-walled carbon nanotubes (MWCNTs). The measurement sensitivity based on CV technique using 2% MWCNTs/ ZnO modified electrode yield as high as 0.009 and 0.082 μ A.mM⁻¹.cm⁻² for ascorbic acid and glucose, respectively.⁸ Albeit the promising and successful detection of bio compounds, the sensitivity remains low for such an electrochemical detection system. Therefore, further effort to increase the measurement sensitivity is proposed by incorporating polymer, which is a commonly used strategy in biosensors to increase the number of target compounds adsorbed on the sensor.

For this purpose, chitosan, a linear polysaccharide composed of β (1-4)-linked N-acetyl-2-amino-2-deoxy-D-glucose, is considered favorable biopolymer for biosensor.¹¹

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Chitosan is known for its good biocompatibility with various bio compounds, and its reactive amino and hydroxyl groups enable chelation various transition metal ions.^{11,12} This attempt, i.e., chitosan modified electrode, has been proven improving the sensing ability: composites of Au-doped chitosan modified electrode enhance the detection limit of glucose and cholesterol (up to 37.89 mg.dL⁻¹).^{13,14} Further effort to improve the measurement sensitivity and specificity has been achieved by introducing conductive materials, such as, graphite, carbon nanotubes, and graphene oxide, in chitosan-based composite to detect other bio-substance, e.g., dopamine.¹⁵⁻¹⁷

Inspired by the above-mentioned strategies, the study at hand presents the development of a new design of a paper-based electrochemical biosensor, whose working electrode is modified using chitosan/ graphene oxide composites. The morphology, microstructure, and electronic properties of chitosan/ graphene oxide composites with different amounts of graphene oxide are characterized. The electrochemical response of the designed biosensor is assessed using impedance spectroscopy and CV. Finally, the performance of biosensor for glucose and paracetamol sensing is evaluated.

MATERIALS AND METHOD

Chemicals, Materials, and Preparation of Chitosan/ Graphene Oxide Composite

Chitosan was prepared using shrimp shells as starting materials. The shrimp shells were washed, boiled, dried, and grounded to remove soluble organics, proteins, and to break the crystal structure of chitin. Demineralization was carried out by using a 7% HCl solution at room temperature for 24 hours. Subsequently, alkali treatment was done using a 10% NaOH solution at 60°C for 24 hours for deproteinization and 50% NaOH solution at room temperature for 3 hours for deacetylation. Additionally, the decoloration of chitosan was introduced by using 0.3% NaOCl solution for 10 minutes. Chitosan/ graphene oxide composite was prepared by mixing the chitosan solution (in diluted citric acid solution) and the graphene oxide (Ossila Ltd., UK) with a certain weight ratio, i.e., 0, 0.5, 1, 2.5, and 5% of graphene oxide. The mixture was first sonicated for 3 hours, and then stirred and irradiated under UV irradiation at room temperature for 4 hours. The paper-based electrode decorated with chitosan/ graphene oxide composites was designed as in Figure 1, which adopted the geometry of the microfluidic channel designed in our previous work.^{18,19} The conductive line was coated with carbon paste prior deposition of chitosan/ graphene oxide. For the reference electrode, silver nanoparticle was deposited on top of one conductive port.

Characterization

Scanning electron micrographs of chitosan/ graphene oxide modified paper electrodes were measured by scanning electron microscope (SEM, FEI type Inspect 21) operated at 100 kV accelerating voltage. XRD patterns were measured using a PAN analytical type X'Pert Pro diffractometer with Cu-K α as the radiation source operated at 40 kV and 40 mA. Diffraction

patterns were scanned at angle (2 θ) between 5 and 40° with 0.05° resolution. FTIR spectra were collected using the Thermo Scientific Nicolet iS10 spectrometer within the range of 400 to 4000 cm⁻¹.

Electrochemical Sensing of Glucose and Paracetamol

The sensing of bio compounds was carried out in the simulated urine. The simulated urine was prepared by CaCl₂·2H₂O (0.28 g), NaCl (0.73 g), Na₂SO₄ (0.56 g), KH₂PO₄ (0.35 g), KCl (0.40 g), NH₄Cl (0.25 g), and urea (6.25 g) in 250 mL distilled water.²⁰ The concentrations of glucose and paracetamol investigated in this study were 2.5, 5, 10, 25, 50, and 100 μ M. For electrochemical measurements, CV and electrochemical impedance spectroscopy were employed. The counter electrode was a carbon electrode, and the reference to oxidation potential was the Ag electrode. The current-voltage response of the CV measurement and the impedance spectroscopy were carried out using Versastat II potentiostat/galvanostat aided with frequency response analyzer.

RESULTS AND DISCUSSIONS

The geometrical design of the chitosan/ graphene oxide modified paper-based electrode is depicted in Figure 1. The sensor is designed for electrochemical sensing, and hence, has three main components, including the working electrode, reference, and counter electrode. Each electrode constitutes a conductive carbon line, whose sheet resistance is 33 \pm 4 Ω .sq⁻¹. The working electrode is in the center of geometry, while Ag-coated carbon (27 \pm 2 Ω .sq⁻¹) and carbon electrode, serve as a reference and counter electrode, respectively.

The physical morphology of chitosan/graphene oxide modified electrode is depicted in Figure 2. SEM images show the qualitative structure of the modified electrode surface. The pristine chitosan film (Figure 2a) shows a rough surface indicated by more number of granules/ agglomerates on the surface. This is plausible as the long chain of chitosan in

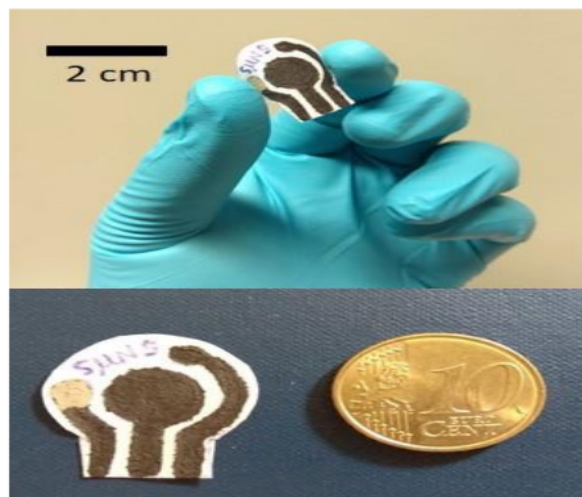


Figure 1: Photograph of chitosan/ graphene oxide modified paper-based electrode

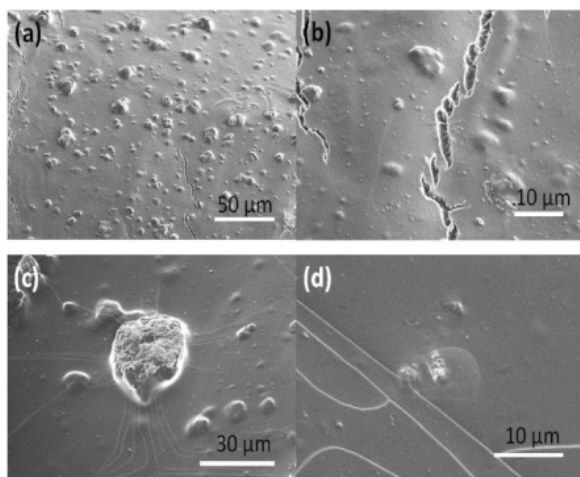


Figure 2: Scanning electron micrograph of chitosan/ graphene oxide surface using different fraction of graphene: (a) 0, (b) 0.5, (c) 2.5, and (d) 5%

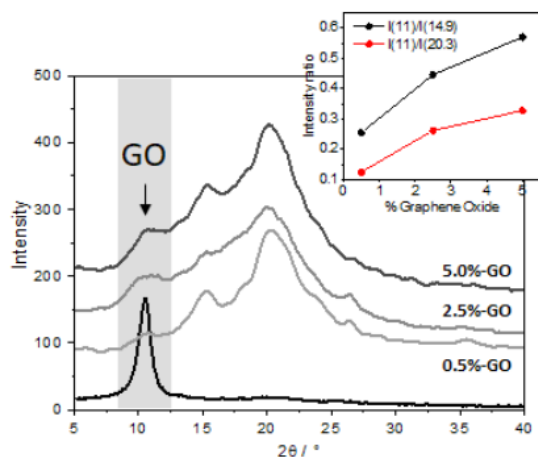


Figure 3: (a) Scanning electron micrograph (SEM), and (b) particle size distribution of WO_3 ; SEM images and BET N_2 adsorption-desorption curve of WO_3/TiO_2 nanocomposites with different compositions (c,d) 1:1 and (e,f) 1:3

solution might promote self-aggregation. Incorporation of graphene oxide smoothens the surface of chitosan/graphene oxide composite films (Figures 2b-d). The tendency of self-aggregation in pure chitosan is likely intercepted due to the cross-linking reaction between chitosan and graphene oxide. This cross-linking is further assessed using vibrational spectroscopy (*vide infra*).

1 Microstructural properties of chitosan/ graphene composites with different loading of graphene was assessed using XRD analysis. The diffraction pattern of either graphene and chitosan/ graphene is displayed in Figure 3. The characteristic diffraction peak of graphene oxide appeared at 2θ of 11° . All of the prepared films exhibit two peaks at 14.9° and 20.3° , which can be assigned to the amorphous state of

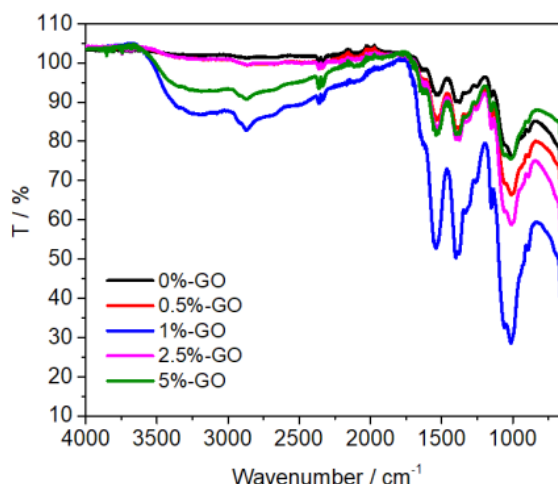


Figure 4: FTIR of chitosan/graphene oxide composites employing different loading of graphene oxide

the chitosan films.^{21,22} The diffraction peaks associated with chitosan is broadened upon addition graphene oxide, while the diffraction peak corresponding to graphite oxide becomes less visible. The intensity ratio of a diffraction peak at 11° to both 14.9° and 20.3° , increases with increasing fraction of graphene oxide (inset Figure 3). This indicates that the crystallinity of chitosan is deteriorated.²² In addition, the broadened peak of amorphous chitosan, as well as, a slight shift of the peak, reflects the molecular structure of chitosan undergoes chemical modification upon compositing with graphene oxide.

The electronic nature of both chitosan and chitosan/ graphene oxide composites is assessed in film form using vibrational spectroscopy, i.e., FTIR spectroscopy (Figure 4). IR spectra of pristine chitosan show a broad and less intense band at $3,100$ to $3,600\text{ cm}^{-1}$, which corresponds to N-H and O-H stretching vibration. The IR band appeared at $2,850\text{ cm}^{-1}$ is attributed to the CH_2 stretching groups. Two characteristic absorption bands centered at $1,640$ and $1,528\text{ cm}^{-1}$ are observed, which can be ascribed to the C-O stretching vibration of NHCO (amide I), and the N-H bending of NH_2 , respectively. In addition, the degree of deacetylation of chitosan here, which is determined from the absorption ratio at $1,640/3,450$, is considered high (96%).

The incorporation of graphene into chitosan alters some IR characteristics. Absorption bands at $1,080$, $1,375$, and $1,634\text{ cm}^{-1}$ for all chitosan/ graphene composites can be ascribed to the C-O-C stretching vibrations, C-OH stretching, and C-C stretching mode of the sp^2 carbon, respectively.^{23,24} Interestingly, the IR band at $3,440\text{ cm}^{-1}$ becomes stronger for 1%- and 5%-graphene contents. Furthermore, it can be seen that the intensity of C-O stretching of chitosan is debilitated, and the absorption band associated with N-H bending vibration of NH_2 is shifted to higher energy (from $1,528$ to $1,542\text{ cm}^{-1}$). The shift is apparently due to the cross-linking reaction between the amino group of chitosan and the epoxy group of

graphene oxide,²⁵ which yields smoother surface in chitosan/graphene oxide composite film.

Prior to use as biosensor, the chitosan/graphene oxide modified paper electrode was subjected to impedance measurement for investigating the electrochemical response toward the target bio compound. An example of electrochemical impedance spectra collected for chitosan/graphene oxide (2.5%) modified electrode, which includes the Nyquist and Bode phase plot, is depicted in Figure 5.

The Nyquist plot show two semicircles at high (> 1 kHz) and medium frequency (< 100 Hz). The semicircle at medium frequency characterizes the charge transfer process at the carbon/chitosan/graphene oxide interface in contact with glucose in simulated urine. As shown, the second semicircle of the Nyquist plot decreases in amplitude, indicating the charge transfer resistance, as well as, the capacitance decrease due to enhanced current flow at the interface.

In addition, the phase in the mid-frequency region is shifted to a higher frequency (f) upon increasing the concentration of glucose. This reflects that the electron transfer process takes place faster ($k = \tau^{-1} = 2\pi f$) for sensing 100 μM glucose than that for lower glucose concentration. This preliminary test is essential to give proof of the concept that the designed chitosan/graphene oxide modified paper electrode is electro-analytically active.

Further evidence is based on the CV measurements, which later are used to determine the sensing quality. It is known that scan rates affect the current response in the CV measurements using the chitosan/graphene oxide modified electrode (Figure 6). Overall, both reduction and oxidation processes at the chitosan/graphene oxide composites yield symmetric anodic and cathodic peaks irrespective of the scan rates. Evaluating the current response (cathodic scan) toward the scan rate results in a linear fit of $I_{pc} = 0.179 + 0.013$ ($r = 0.9994$). This linear relationship follows the equation:

$$I_p = nFQv/4RT$$

where n is the number of charges, F is Faraday constant, Q is the total charge, v is the scan rate, R is the universal gas constant, and T is the temperature. If the peak current response is normalized with the scan rate, it will yield a similar total charge amplitude, which indicates that the redox reaction of glucose on the chitosan/graphene oxide modified electrode is a quasi-reversible surface dictated electrochemical process.²⁶

The electrochemical parameter for glucose and paracetamol sensing is extracted from CV. The current response (anodic scan) are recorded for various glucose (Figure 7) and paracetamol (Figure 8) concentration in simulated urine, which ranges between 2.5 and 100 μM . Irrespective of graphene oxide content in chitosan/graphene oxide modified electrode,

the current responses show a linear fit ($R^2 = 0.99$) toward glucose and paracetamol concentration (C). In addition, the measurement's sensitivity (k), which is indicated by the slope in linear fit $y = k.C + y_0$, constantly increases upon increasing graphene oxide content in the modified electrode. This indicates that the addition of graphene oxide improves conductivity due to its higher inherent electrical conductivity,^{27,28} and hence, increases the electrochemical detection using CV.

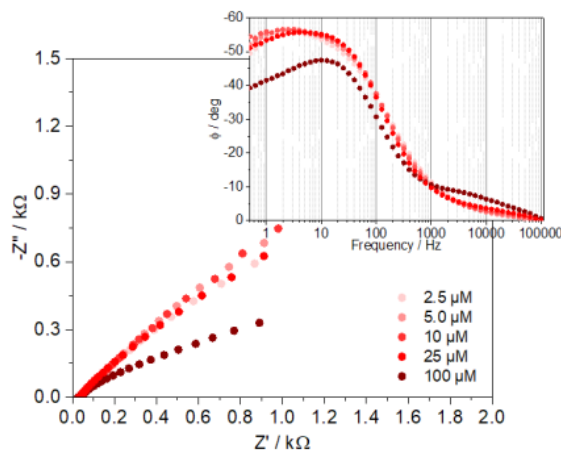


Figure 5: Impedance spectra of chitosan/graphene oxide (2.5%) modified electrode for glucose measurement represented in Nyquist and (inset) Bode plot

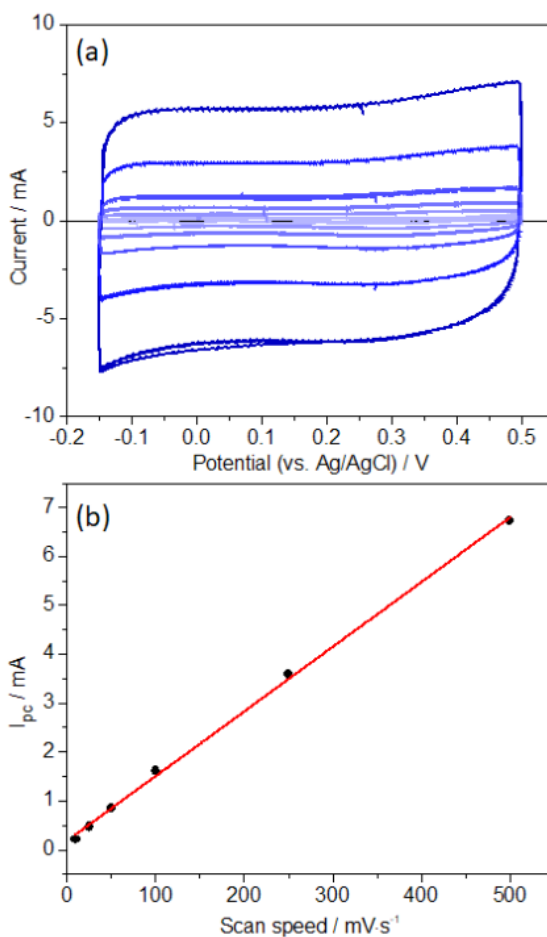


Figure 6: (a) Cyclic voltammograms (CV) of 100 μM glucose were measured using chitosan/graphene oxide (2.5%) modified electrode at different scan rates; (b) Current response vs. scan rate plot derived from CVs

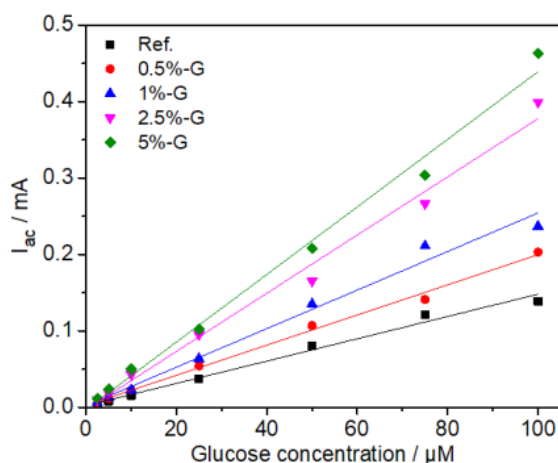


Figure 7: Current response measurement of glucose using chitosan/graphene oxide modified paper electrodes

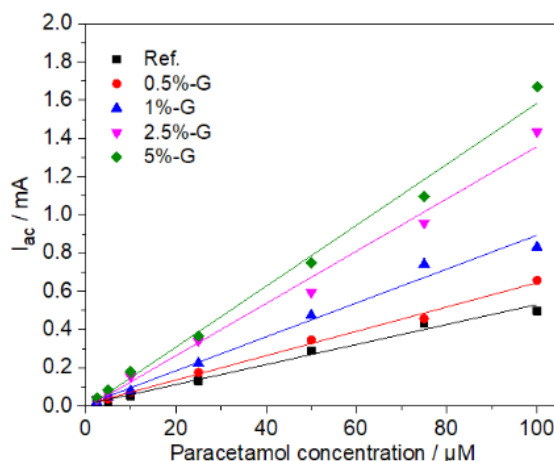


Figure 8: Current response measurement of paracetamol using chitosan/graphene oxide modified paper electrodes

For glucose measurement, the sensitivity increases from 1.45 to 4.42 mA.mM⁻¹, with increasing the graphene content from 0 to 5%. Compared with the sensitivity reported in the literature on electrochemical sensing of glucose using chitosan/graphene composite modified with either glucose oxidase (37.93 A.mM⁻¹) or Fe₃O₄ (5.66 mA.M⁻¹), the result at hand is indeed inferior.^{26,29} Nonetheless, it is still promising since another graphene oxide based sensor only exhibits a sensitivity of 10 µA.mM⁻¹.³⁰ For glucose measurement, the sensitivity increases from 1.45 to 4.42 mA.mM⁻¹, with increasing the graphene oxide content from 0 to 5%.

In line with results obtained for glucose measurement, electrochemical paracetamol sensing shows relatively higher sensitivity, which increases from 5.24 to 15.95 mA.mM⁻¹ with increasing the graphene content from 0 to 5%. The highest sensitivity for paracetamol detection here is found better than electrochemical sensing of paracetamol reported in the literature using N-doped multi-walled carbon nanotubes electrode with a sensitivity of 0.841 A.M⁻¹.³¹

CONCLUSION

We have developed a paper-based electrochemical biosensor that is functional for glucose and paracetamol measurements. The working electrode of the paper biosensor was modified by using cross-linked chitosan/graphene oxide composites and successfully demonstrated electroanalytic character to detect glucose and paracetamol in simulated urine. CV-based measurements indicate an increasing trend in the sensitivity of biocompounds detection upon increasing the graphene oxide concentration in the composite. For chitosan/graphene oxide (5%) composites, the sensitivity of the device is found as high as 4.42 and 15.95 mA.mM⁻¹ for glucose and paracetamol sensing, respectively. A constantly increasing sensitivity obtained in this study demands further exploration of the chitosan-based composite with the concentration of graphene oxide beyond 5%.

ACKNOWLEDGMENT

This work was financially supported by research grant “Penelitian Pemula” No. 1223/PKS/ITS/2019 by Lembaga Penelitian dan Pengabdian Masyarakat of Institut Teknologi Sepuluh Nopember.

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