A facile electrochemical sensor based on composite of electrochemically reduced graphene oxide and PEDOT:PSS modified glassy carbon electrode for uric acid detection

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Abstract

A glassy carbon electrode modified with electrochemically reduced graphene oxide (ErGO) and poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) was developed for the uric acid determination with dopamine as interference in artificial saliva. The electrochemical performance of the fabricated electrode was studied using both techniques of cyclic voltammetry (CV) and differential pulse voltammetry (DPV), under optimized conditions. Using DPV, the sensor based on ErGO/PEDOT:PSS modified GCE displayed a linear relationship in the concentration ranges of 10-100 μM for uric acid. This uric acid sensor exhibited a high sensitivity with the detection limits of 1.08 μM and the quantitation limit of 3.61 μM . This sensor also showed good reproducibility for uric acid detection in the artificial saliva in 5 consecutive days measurements. This device was successfully used to analyze uric acid in the artificial saliva as well as in human saliva sample using standard addition method.

Keywords electrochemically reduced graphene oxide, PEDOT:PSS, uric acid, dopamine, artificial saliva

Introduction

Uric acid (2,6,8-trihydroxypurine) is an important final product of metabolic purine and mainly dissolves in human blood, plasma, urine, and saliva. In the healthy human, the uric acid concentration in the saliva normally in the range of $199 \pm 27 \mu\text{mol/L}$ and comparable observed in the serum approximately to $120 - 400 \mu\text{mol/L}$.¹ Previous studies have reported the linear correlation of uric acid concentration in human body both in the blood serum and salivary.²⁻⁴ The high concentration of uric acid are symptoms to several disorders in the human body such as Lesch-Nyhan syndrome, kidney disease, gout, hypertension, hyperuricaemia, atherosclerosis,

Alzheimer disease, Parkinson disease, and other diseases.⁵⁻⁷ Therefore, an assay preferably rapid, simple, and reliable is essential to be developed for uric acid determination to monitor these diseases in the human physiological fluids.

Previously, various techniques have been employed to determine the uric acid concentration such as gas chromatography-mass spectrometry⁸, high-performance liquid chromatography⁹⁻¹⁰, UV-Vis spectrophotometry¹¹, chemiluminescence¹², fluorescence.¹³ However, these methods are reported complex to perform, time consuming, laborious, and requires expensive equipment thus impractical for daily analysis. Among these techniques, electrochemical method has offered an alternative approach for uric acid determination due to its simplicity, rapid detection, low cost, and need only a less amount of sample for routine analysis. Up until now, there are many works have been performed to investigate the electrochemical detection of uric acid determination using different modified electrodes.¹⁴⁻²² However, yet still it is potentially prospective to develop a facile and low cost of an electrochemical sensor for uric acid detection with high sensitivity and selectivity.

The sensitivity of an electrochemical sensor for uric acid detection can be improved by utilizing modifiers acts as electrocatalyst e.g. metal nanoparticles²³⁻²⁴ and carbon nanomaterials.²⁵⁻²⁶ Among them, reduced graphene oxide (rGO) due to its benefits such as easy to modify, good chemical stability and electrochemical activity with high conductivity, it has lately emerged as a novel material for the electrode modifier.^{27,28} However, the actual capability of rGO materials to be used as a modifier electrode might be reduced due to an accumulation within the graphene layers. This effect is attributed to the van der Waals interaction and $\pi - \pi$ stacking between carbon molecules within graphene layers.²⁹ Several works in the past have been reported the better performance of sensing application when rGO combined with conductive polymer (CPs) such as polyaniline³⁰⁻³¹, polypyrrole³², and p-phenylenediamine.³³ Therefore, it is interesting to combine

the rGO material with CPs to reduce the accumulation phenomena within graphene layer as well as to enhance the electrocatalytic properties of the modified electrode for sensing purposes.

Among CPs, Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) or PEDOT:PSS has been actively investigated to be employed as an electrode modifier due to its high conductivity³⁴, a broad electrochemical window and good chemical stability³⁵, and good film-forming process.³⁶⁻³⁷ The presence of PSS in PEDOT plays an important function for polymer stability also could facilitates an electrostatic interaction towards oxygen-rich functional groups in GO material.³⁸ In addition, the PEDOT:PSS material might perform a suitable barrier to stop the restacking process of rGO layers, and thus enhancing its conductivity, electrocatalytic activity, and specific surface area.³⁹ Therefore, by preparing a homogenous dispersion of rGO with PEDOT:PSS may not only provide a suitable additive to separate rGO layers but also introduces the feasibility to be used as an electrochemical sensor materials.⁴⁰

In this paper, we present a facile approach to combine the GO material with the PEDOT:PSS and drop casted it to the glassy carbon electrode (GCE) surface. Then, GO material was reduced into ErGO by a cyclic voltammetry technique to obtain the composite of ErGO/PEDOT:PSS on the working electrode. The electrochemical performance of ErGO/PEDOT:PSS composite were compared with GO, PEDOT:PSS, GO/PEDOT:PSS, and bare GCE for uric acid detection. The utilization of these electrode modifier as the material sensor for uric acid were then studied and discussed. Based on our knowledge, the utilization of ErGO/PEDOT:PSS composite for uric acid detection with dopamine as interference in the artificial saliva has not been investigated. Thus, this work was focused on the fabrication of ErGO/PEDOT:PSS on the GCE surface and evaluated its performance for uric acid sensor by using a differential pulse voltammetry (DPV) technique. The performance of this uric acid sensor has been evaluated in the presence of dopamine as interference as well in artificial saliva as the matrix solution. The results show that this sensor demonstrated good electrochemical responses for uric acid sensing with high stability and selectivity in the presence of dopamine.

Experimental

Reagents and apparatus

Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS), graphite, NaNO_3 , conc. H_2SO_4 , KMnO_4 , H_2O_2 30%, uric acid (CAS number: 1198-77-2), artificial saliva, dopamine were obtained from Sigma-Aldrich Ltd. and used without further purification. 3 mm diameter glassy carbon electrode was obtained from IJ Cambria Scientific.

Electrochemical experiments were carried out using the PalmSens Emstat 3 potentiostat (ES316U669). Infrared spectrum was obtained from Bruker Fourier Transform Infrared Tensor 37. Raman spectra was obtained from Micro Confocal Hyperspectral 3D Imaging Raman Spectrometer. The standard apparatus in the laboratory such as Pyrex glassware, magnetic stirrer, Eppendorf micropipette, analytical balance (Ohaus Instruments Co. Ltd.), and oven (Mettler GmbH) were used during the experiments.

Graphene oxide synthesis (modified Hummer's method)

Graphene oxide was synthesized by following the modified Hummer's protocol. Firstly, graphite (1 g) and NaNO_3 (0.5 g) was added into concentrated H_2SO_4 (25 mL) and the mixture was cooled to 0 °C. Then, the mixture was stirred kept at constant temperature of 0 °C for 1 hour. Next, KMnO_4 (3 g) was slowly added and the temperature was maintained below 20 °C. The mixture was stirred for 1 hour and left for 30 minutes at the room temperature. Next, 50 mL of water was added into the mixture which produce an exothermic reaction to 90-95 °C. Then, the resulted mixture was stirred for 1 hour and left 15 minutes afterwards. The reaction mixture was terminated by an addition of 50 mL of H_2O_2 30%. Next, the mixture was stirred for 1 hour and cooled afterwards. Finally, the reaction mixture was washed with water to remove the metal ions and then dried. Characterization of the obtained GO was characterized by Raman and Fourier Transform Infrared (FT-IR) Spectroscopy.

Modification of GCE and GO reduction process on the GCE surface

Each of GO and PEDOT:PSS was diluted to 1 mg mL⁻¹ with deionized water. On the other hand, 1 mg of PEDOT:PSS was mixed with 1 mg of GO into a vial and diluted with deionized water to obtain the composite solution of GO/PEDOT:PSS 1 mg mL⁻¹. All of solutions were then sonicated for 1 hour. To modify the GCE surface, each of 4 µL of GO, PEDOT:PSS, GO/PEDOT:PSS was drop casted onto the surface of glassy carbon electrode (GCE). The modified GCE was then kept in the oven at 100 °C for 5 minutes to obtain a stable film.

The GO/PEDOT:PSS film was then reduced by potential cycling between 0 and -1.3 V at the scan rate of 50 mV s⁻¹ in 0.1 M KCl to obtain electrochemically reduced graphene oxide (ErGO) modified PEDOT:PSS (ErGO/PEDOT:PSS). Then, the reduction process of GO on the GCE surface was optimized to be used in the measurement of uric acid. First parameter for the optimization of GO reduction is the number of cyclic process which is varied from 10 to 40 cycles. The optimum cycle number was evaluated based on the oxidation current from cyclic voltammogram of 200 µM uric acid in 0.1 M KCl (scan rate, 100 mV s⁻¹) at the modified electrode. The scan rate of GO reduction process was also varied at 25, 50, 75, 100, and 125 mV s⁻¹ and evaluated in the same manner as the number of cycles. From this investigation, it was obtained the optimum condition of GCE modified ErGO/PEDOT:PSS further to be used in the performance characterization of uric acid measurements. Fig. 1 shows the step-by-step illustration of fabrication and modification process of glassy carbon electrode (GCE) with ErGO/PEDOT:PSS.

Evaluation of analytical performance of uric acid measurement

Linearity, Limit of Detection and Limit of Quantitation. Linearity was evaluated by the preparation of uric acid solution in different concentrations in range of 10 to 100 µM in 0.1 M

artificial saliva. Each of concentration was scanned using differential pulse voltammetry technique in triplicates at potential window of 0 to +1V, scan rate of 25 mV s^{-1} , potential step of 5 mV, potential pulse of 25 mV, and pulse time of 0.1 s. Linearity was determined from the relationship between solution concentration with current response of uric acid. Limit of Detection and Limit of quantitation were evaluated based on calibration curve of three replicates.

Repeatability and Stability. Electrode repeatability was evaluated by preparing 5 different electrodes of GCE modified ErGO/PEDOT:PSS. The repeatability test was performed by measuring $100 \text{ }\mu\text{M}$ uric acid solution in the artificial saliva using those 5 electrodes. On the other hand, electrode stability was also evaluated by measuring $100 \text{ }\mu\text{M}$ uric acid solution in the artificial saliva using the same electrode for 5 consecutive days. All electrochemical measurement was performed using differential pulse voltammetry technique at potential window from 0 until +1V, scan rate of 25 mV s^{-1} , potential step of 5 mV, potential pulse of 25 mV, and pulse time of 0.1 s.

Simultaneous determination of uric acid and dopamine. Uric acid and dopamine were prepared in a single solution with the concentration of uric acid twice than dopamine. The solution was prepared in a series of concentrations as follow for uric acid as 40, 60, 80, 100, 120, 140 μM and dopamine as 20, 30, 40, 50, 60, and 70 μM in the artificial saliva. Each of concentration was scanned with differential pulse voltammetry technique in triplicates at potential window of -0.05 to +0.5 V, scan rate of 25 mV s^{-1} , potential step of 5 mV, potential pulse of 25 mV, and pulse time of 0.1 s.

The determination of uric acid in the artificial saliva using GCE modified ErGO/PEDOT:PSS compared with a commercial uric acid sensor.

Uric acid was prepared in a series of concentrations as follow 160, 170, 180, 190, and 200 μM in the artificial saliva. Each of concentration was scanned with differential pulse voltammetry in triplicates at potential window from 0 until +1V, scan rate of 25 mV s^{-1} , potential step of 5 mV, potential pulse of 25 mV, and pulse time of 0.1 s. The linear relationship between uric acid concentration with current response was obtained from this experiment. Then, 180 μM of uric acid solution was measured using the modified GCE and a commercial uric acid sensor device. The concentration of uric acid obtained from the modified GCE was calculated using the calibration curve and compared with a commercial uric acid sensor.

Measurement of uric acid in human saliva using GCE modified ErGO/PEDOT:PSS.

The sample of human saliva was collected from healthy volunteer. The collected samples of saliva were directly used for DPV measurements without any further treatment. Briefly, 1 mL of saliva sample was spiked with uric acid standard solution and diluted into 10 mL using electrolyte to obtain the final concentration of spiked uric acid in the range of 0 – 100 μM . The obtained saliva samples were then measured with DPV technique under same experimental conditions. The concentration of uric acid in human saliva sample was calculated based on the x-intercept of calibration curve of standard addition.

Results and Discussion

It is well known that with the presence of reducing agent i.e. NaBH_4 , the composite film of GO/PEDOT:PSS will both reduced to rGO and non-conducting form of PEDOT:PSS.⁴¹ The reduction method using reducing agents causes removal of all oxygen in the GO chemical

structures and irreversible degradation process of PEDOT:PSS backbone.^{42,43} A milder reduction method via the electrochemical reduction of composite film offers an alternative approach due to its simplicity and safer compared to the chemical reduction of GO. In addition, by using the electrochemical methods, there is no loss of PEDOT:PSS electroactivity since it is reversible process while GO reduction is known to be irreversible.⁴¹ The electrochemical reduction of GO in the composite of GO/PEDOT:PSS film was performed using cyclic voltammetry technique by applying 0 to -1.3 V at 50 mV s⁻¹ for 40 cycles. The cyclic voltammogram in Fig. 2a shows a cathodic current indicated the beginning process of the electrochemical reduction of GO which started to appear at approximately -0.4 V.⁴⁴ This cathodic current was due to the reduction process of the electroactive oxygen functional groups in the GO/PEDOT:PSS film. By repeating the cycling process, both of cathodic and anodic currents increased linearly up to 40 cycles which suggests the increasing of reduction process of GO material. The changes of CV shapes describe an enhancement of non-Faradaic currents due to an increasing of the effective surface area that are generated by GO reduction leading to electrically conductive graphene material.⁴⁵ The general features of CV curves obtained in Fig. 2a indicating that the graphene oxide in the GO/PEDOT:PSS film has been successfully reduced to the reduced GO on the surface of glassy carbon electrode.

Characterization by Raman Spectroscopy and FT-IR

Fig. 2b shows the Raman spectra of graphite, graphene oxide (GO), and composite of electrochemically reduced graphene oxide and PEDOT:PSS (ErGO/PEDOT:PSS). The typical Raman spectrum of carbon-based materials contains prominent features i.e. D peak (1345 cm⁻¹), G peak (1585 cm⁻¹), and 2D peak (2700 cm⁻¹).⁴⁶ The D band corresponds to the existence of defects in the graphite material which can be correlated to the vacancies, dislocations, or cracks in the graphene layer.⁴⁷ The G peak is attributed to the in-plane vibrations of sp²-hybridized

carbon atoms constituted the graphene sheets in the structure of graphite material.⁴⁸ While the last peak (2D) denotes the number of graphene layers and could be used as a Raman fingerprint analysis in the carbon-based materials.⁴⁹ In the Raman spectra of graphene oxide and its composite, two main peaks were clearly visible at 1345 cm^{-1} (D peak) and 1585 cm^{-1} (G peak). The removal of oxygen groups during the reduction process of GO generated an enhancement in the out-of-plane sp^2 vibrations in the graphene structure.⁵⁰ In addition, when PEDOT:PSS were mixed with reduced graphene oxide (ErGO), the PEDOT polymer was incorporated to the ErGO surface via the interaction of π - π stacking. This new interaction between PEDOT:PSS and ErGO material forms a well-defined coated film on the graphene surface.⁵¹ Therefore, it is interesting to further characterized the composite of ErGO/PEDOT:PSS using FT-IR technique and also to examine the electrochemical performance of its electrode to be used as an uric acid sensor.

FT-IR spectroscopy was used to confirm the removal of oxygen functional groups from GO structure after electrochemical reduction process. Fig. 2c shows the IR spectra for both of GO and electrochemically reduced GO (ErGO). The spectrum for GO film shows the vibration peaks at 1050 cm^{-1} (C-O stretching), 1580 cm^{-1} (C=C stretching), 1750 cm^{-1} (C=O stretching), and a broad peak at 3500 cm^{-1} (O-H stretching). After the electrochemical reduction process to GO, the intensity of vibration peaks at 1050, 1750, and 3500 cm^{-1} were declined, indicating the elimination of oxygen functional groups in GO film. The vibration of functional groups of PEDOT:PSS and ErGO/PEDOT:PSS films were also characterized by FT-IR spectra and shown in Fig. 2c It can be seen that both IR spectra contain vibration peaks at 1650 cm^{-1} (C=C stretching), 1510 cm^{-1} (C=C aromatic stretching), and 1300 cm^{-1} (C-C stretching) corresponds to the thiophene backbone in PEDOT:PSS structure. In addition, the peaks at 1200 cm^{-1} (C-S stretching) in the EDOT structure, 990 cm^{-1} and 850 cm^{-1} linked to the S-O bands in the PSS structure.

248 *The electrochemical oxidation of uric acid at the ErGO/PEDOT:PSS modified GCE*

249 The electrochemical behaviors of uric acid were evaluated using the bare GCE and the
250 modified electrode in the potential range from 0 to 1.0 V. Fig. 3a shows the cyclic voltammograms
251 obtained from GCE, PEDOT:PSS/GCE, GO/GCE, GO-PEDOT:PSS/GCE, and ErGO-
252 PEDOT:PSS/GCE for 200 μ M uric acid in 0.1 M KCl at the oxidation potential of 0.55 V vs
253 Ag/AgCl. The absence of reduction peaks during the scanning process towards negative potential
254 (cathodic) illustrated that the uric acid went through an irreversible oxidation reaction. The anodic
255 peak current of uric acid at ErGO/PEDOT-PSS shows more than 12 times higher than at GCE
256 reflecting a significant electrocatalytic activity of ErGO/PEDOT:PSS film. The enhanced
257 electrocatalytic activity of ErGO/PEDOT:PSS can be associated with providing a large surface
258 area and a highly conductive film adherent to the GCE surface. This resulted a significant
259 improvement of the electron transfer process between the modified electrode and uric acid
260 compared to the oxidation current on the bare GCE. The immobilization of PEDOT:PSS modified
261 reduced GO on the GCE surface forming a homogenous film with good adhesion property.⁵² The
262 mechanism of this process was due to the hydrogen bonding between the remaining oxygen
263 functional groups of reduced GO with sulphonate groups on the hydrophilic side in PEDOT:PSS
264 contributed to the enhanced conductivity of the composite film formation.⁵³ The synergistic effect
265 between PEDOT:PSS and reduced GO makes them as an outstanding platform for the uric acid
266 sensing through electrocatalytic oxidation processes.

267 In order to determine the optimum reduction process for ErGO/PEDOT:PSS film
268 preparation, GO/PEDOT:PSS film was electrochemically reduced in the electrolyte of 0.1 M KCl
269 with potential cycling at 50 mV s⁻¹ (from 0 to -1.3 V) in different number of cycles. Fig. 3b shows
270 the current response obtained from measuring 200 μ M uric acid after reduction process of
271 GO/PEDOT-PSS film in 0.1 M KCl with different number of cycles (10, 15, 20, 25, 40). It can
272 be seen with the increasing number of cycles for the reduction of GO/PEDOT-PSS film, the

current response from uric acid oxidation was also improved. This result indicates that the removal of the certain functional groups from GO structures such as -OH, -C=O, -COOH leading to the ErGO/PEDOT:PSS film formation on the electrode. This also reflects that the formation of ErGO/PEDOT:PSS film provides a highly conductive substrate on the GCE surface generating an improvement of electron transfer between the electrode and uric acid. However, we chose 40 cycles as the selected parameter in cyclic voltammetry technique for the electrochemical reduction process of GO/PEDOT:PSS film for subsequent electrode modification. Fig. 3c shows the optimum scan rate for reduction process of GO/PEDOT:PSS film evaluated through measurement of 200 μ M uric acid in the electrolyte of 0.1 M KCl. The results shows that ErGO/PEDOT:PSS film obtained from reduction process at scan rate of 50 mV s^{-1} provided higher oxidation current of 200 μ M uric acid. This scan rate (50 mV s^{-1}) was chosen for the subsequent of GO electrochemical reduction process to obtain ErGO/PEDOT:PSS.

ErGO/PEDOT:PSS modified GCE was then used for uric acid measurement at different scan rate. Cyclic voltammogram of uric acid at ErGO/PEDOT:PSS modified GCE with different scan rates ranging from 25 to 250 mV s^{-1} . The peak current of I_{pa} displayed a linearly relationship to the square root of the scan rate and the obtained correlation coefficients is 0.9945 (Figure not shown), indicating that the oxidation process of uric acid is diffusion controlled. In addition, as the scan rate increased, the peak potential of anodic reaction slightly shifted to more positive values, confirming the irreversible reaction process of the uric acid oxidation.

Electrochemical evaluation of the ErGO/PEDOT:PSS modified GCE for uric acid detection in the artificial saliva

The electrochemical performance of ErGO/PEDOT:PSS modified GCE for uric acid sensing was initially studied in the artificial saliva containing uric acid at different concentrations (10 – 100 μ M). The DPV technique was employed in this study due to its higher sensitivity than cyclic

voltammetry and amperometric methods. As shown in Fig. 4a, it shows well-defined peaks that positively increased with increasing of the uric acid concentration from 10 – 100 μM in the artificial saliva proportionally to the oxidation peak currents. The potential oxidations of uric acid appear approximately at 160 mV vs Ag/AgCl in the artificial saliva. The equation for linear regression between oxidation current against different concentrations of uric acid is expressed as $I (\mu\text{A}) = 0.1564 (\mu\text{M}) - 0.5698$ ($R^2 = 0.9925$). Each point is an average derived from triplicates in the experimental works. From this works, it was obtained the both value of detection limit and quantification limit as 1.08 μM (0.18 $\mu\text{g mL}^{-1}$) and 3.61 μM (0.606 $\mu\text{g mL}^{-1}$), respectively. The value of detection limit is lower than uric acid concentration in normal human saliva indicated that this sensor is sensitive to uric acid and potentially could be used in human saliva sample. Also, the other main parameters of this sensor such as the concentration range and limit of quantification in this work were also comparable with those obtained by other uric acid sensors, as summarized in Table 1. The comparable or improved performance of uric acid sensor based on the ErGO/PEDOT:PSS modified GCE is ascribed to the good electrocatalytic performance of ErGO/PEDOT:PSS. From Table 1, it can be seen that this proposed sensor has lower limit of detection compared to other uric acid sensor based on homemade SPCE.^{16,54} Moreover, other GCE based modified nanocomposites showed superior performance in terms of its detection limit and quantitation limit.⁵⁵⁻⁵⁷ But this uric acid sensor displayed a wider linear range of concentrations contrastingly to other modified electrodes such as homemade SPCE.^{16,18,58} The benefit of the present uric acid sensor includes the low of detection limit and quantitation limit as well as wide linear range of concentration which are attributed to the synergistic effect of reduced graphene oxide and PEDOT:PSS.

The reproducibility of electrode in terms of the fabrication and day to day response has been evaluated due to its crucial parameter as a disposable uric acid electrochemical sensor. The reproducibility of the proposed uric acid sensor was studied using 5 different electrodes fabricated separately using the similar method and the same electrode has been used in 5 consecutive days.

DPV measurements of 0.1 mM uric acid in the artificial saliva samples yielded the value of relative standard deviation (RSD) of 2.87% using 5 different electrodes and 2.27% along the 5 consecutive days measurements. These results indicate that the proposed sensor based on ErGO/PEDOT:PSS modified GCE provides a good reliability to be used in the artificial saliva.

Simultaneous detection of uric acid and dopamine at ErGO/PEDOT:PSS modified GCE

The current response from uric acid measurements in the body fluids are generally hampered due to the presence of interference such as e.g. dopamine. Thus, it is interesting to investigate the effect of interference when the uric acid concentration as analyte was adjusted two times higher than the dopamine in the artificial saliva. The principle of measurement was based on the simultaneous measurements of uric acid and dopamine in the artificial saliva and each of its electrochemical oxidation reaction process depicted in Scheme 1. As shown in Fig. 4b, the oxidation peaks of dopamine and uric acid appear at approximately 40 mV and 140 mV in artificial saliva with their increasing concentrations. The oxidation potentials of uric acid are slightly shifted into more negative (140 mV) in the matrix containing dopamine due to its viscosity and the complex composition.⁵⁹ The consecutive concentrations of dopamine and uric acid in the artificial saliva give linear relationship towards the oxidation currents as a function of concentration. The obtained calibration equation was $I (\mu\text{A}) = 0.0227 (\mu\text{M}) + 0.4557$ with a calibration coefficient of 0.9076 and $I (\mu\text{A}) = 0.0441 (\mu\text{M}) + 0.2378$ with a calibration coefficient of 0.9907 for dopamine and uric acid, respectively. It can be concluded that these results indicated that the simultaneous determination of two species in the artificial saliva using DPV method are feasible using the ErGO/PEDOT:PSS modified GCE.

Comparison of uric acid detection at ErGO/PEDOT:PSS modified GCE and using commercial sensor

To assess the validity of the proposed modified electrode, the estimation of uric acid concentration was also compared using commercial sensor in the artificial saliva. The commercial uric acid sensor used for validation was multipurpose monitoring system of blood glucose, cholesterol, and uric acid. The real concentration of uric acid in artificial saliva was 180 μM and when it was measured using commercial sensor as 220 μM . However, when the uric acid in the artificial saliva was measured with the proposed modified electrode obtained 178.64 μM . This result displays that the proposed modified electrode yields a good accuracy and reliability to be used in the uric acid measurements in the artificial saliva.

The performance of proposed uric acid sensor was then evaluated in the sample of human saliva obtained from healthy volunteer. Fig. 4c shows the response curve of the spiked uric acid in the concentration range of 0 – 100 μM from sample of human saliva. Based on the x-intercept of the spiked calibration curve, it was obtained the average concentration of uric acid from human saliva as 146.05 μM which is in a good agreement with previous studies.⁶⁴

Conclusions

In summary, we have demonstrated a facile strategy to reduced graphene oxide in the GO/PEDOT:PSS composite using the electrochemical technique which was resulted in the significant improvement to be used as material sensor. Further, the material sensor based ErGO/PEDOT:PSS was investigated for uric acid sensing with a low limit of detection of 1.08 μM in the matrix of artificial saliva. The above results show the composite of ErGO/PEDOT:PSS displayed good sensitivity, reproducibility, and selectivity in the presence of dopamine as an interference under optimal working conditions. The performance of the proposed sensor was also

comparable with the commercial uric acid sensor and could be used in the human saliva sample. This work shows that the developed method based on the composite of ErGO with conductive polymers is a promising material for uric acid sensor.

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480 Table 1 Comparison of performance of the proposed uric acid sensor with the other sensors

Technique	Electrode	Linear range / μM	LOD / μM	Ref.
DPV	Homemade SPCE modified PEDOT:PSS	20 – 100	1.62	[16]
DPV	HNGA ^a modified GCE	0.4 – 50	0.12	[18]
CV	SPCE modified carbon black-graphene oxide	0.05 – 2000	0.01	[19]
DPV	Homemade SPCE	10 – 80	1.94	[54]
DP-ASV ^b	ZIF-11 ^c modified GCE	20 – 540	0.48	[55]
DPV	LCP ^d modified GCE	0.020 – 25	2.27×10^{-2}	[56]
DPV	Carbon dots (CD) modified GCE	3 – 75	0.011	[57]
LSV	AuNPs/PANI ^e modified GCE	20 – 100	16	[58]
DPV	Disposable paper modified PEDOT-GO	2 – 1000	0.75	[59]
CV	SPE-AuNPs	20 – 200	11.91	[60]
DPV	CM ^f /AgNPs/SDS ^g /MWCNT ^h /GCE	18 – 650	0.14	[61]
Amperometric	G-SrWO ₄ /Nafion modified GCE	0.001 – 0.5	196.3	[62]
LSV	rGO/AuNPs modified ITO-PET sheets	10 – 500	3.6	[63]
DPV	ErGO/PEDOT:PSS modified GCE	10 – 100	1.08	This work

^a Holey nitrogen-doped graphene aerogel. ^b Differential pulse-adsorptive stripping voltammetry. ^c Zeolite imidazolate framework-11. ^d Lithium cobalt phosphate. ^e Polyaniline. ^f Curcumin. ^g Sodium dodecyl sulfate. ^h Multi-walled carbon nanotubes.

481

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Scheme 1. Electrochemical oxidation reaction of (A) dopamine and (B) uric acid

PEDOT:PSS

Fig. 1 Step-by-step illustration and modification process of glassy carbon electrode (GCE) with ErGO/PEDOT:PSS

490 Fig. 2 (a) Cyclic voltammogram of GO/PEDOT:PSS film on the GCE surface in 0.1 M KCl along
 491 40 cycles reduction processes at 50 mV s^{-1} scan rate, (b) Raman spectra of graphite, graphene
 492 oxide (GO), and composite of electrochemically reduced graphene oxide and PEDOT:PSS
 493 (ErGO/PEDOT:PSS) and (c) Infrared spectrum of graphene oxide (GO), electrochemically
 494 reduced graphene oxide (ErGO), PEDOT:PSS, and composite of electrochemically reduced
 495 graphene oxide and PEDOT:PSS (ErGO/PEDOT:PSS).

Fig. 3. (a) cyclic voltammogram of 200 μM uric acid in 0.1 M KCl (scan rate, 100 mV s^{-1}) at bare GCE, PEDOT:PSS modified GCE, GO modified GCE, GO/PEDOT:PSS modified GCE, and ErGO/PEDOT:PSS modified GCE, and the oxidation current of 200 μM uric acid in 0.1 M KCl measured at ErGO/PEDOT:PSS modified GCE obtained from different reduction condition (b) number of reduction cycles (c) scan rate of reduction cycles.

Fig. 4 (a) DPVs recorded with ErGO/PEDOT:PSS modified GCE containing different concentrations of uric acid (10-100 μM) in artificial saliva. Inset: the linear relationship between peak current the uric acid concentration, (b) DPVs recorded with ErGO/PEDOT:PSS modified GCE containing different concentrations of uric acid (UA) (40-140 μM) and dopamine (DA) (20-70 μM) in artificial saliva, (c) DVPs response of saliva sample spike with the increasing concentration of uric acid in range of 0 – 100 μM , the inset is resulting calibration plot.