

Electrochemical determination of adenine using a glassy carbon electrode modified with graphene oxide and polyaniline

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Abstract The electrochemical behavior of adenine at a glassy carbon electrode (GCE) modified with a nanocomposite consisting of graphene oxide and polyaniline was investigated by cyclic voltammetry and differential pulse voltammetry. The nanocomposite was synthesized by polymerization and characterized by Raman and UV-vis spectroscopy, and its morphology was examined by scanning electron microscopy. Adenine is oxidized at the modified GCE at a working potential of 1.2 V (vs. Ag/AgCl) and gives a current density of approximately $2.64 \text{ nA}\cdot\text{cm}^{-2}$, which is distinctly increased compared to the $0.57 \text{ nA}\cdot\text{cm}^{-2}$ of a bare electrode. Peak current and adenine concentration are linearly related to each other in the range from $0.5 \mu\text{M}$ to $20 \mu\text{M}$. The modified GCE exhibits acceptable analytical performance, with a detection limit of 72 nM and a limit of quantification of 240 nM. It is excellently reproducible, stable, and fabrication is simple.

Keywords Nanocomposite · Sensor · Cyclic voltammetry · Differential pulse voltammetry · Scanning electron microscopy · Raman spectroscopy

Introduction

During the last decade, graphene, a honeycomb two-dimensional lattice, configured with sp^2 carbon atoms and its derivatives, has attained considerable interest thanks to its magnificent structure and properties [1, 2]. Among graphene derivatives, graphene oxide provides a significant potential for sensitive detection of immobilized biomolecules due to its large surface area and high electron mobility through graphene oxide sheets [3]. It is believed that the presence of oxygen moieties on the basal plane of graphene oxide facilitates the interaction with other materials at surface interface and assists to form nanocomposites [4]. Moreover, the electrochemical properties of GO can be easily stimulated by the integration of other functional materials [5]. It has been demonstrated that the conducting polymer/GO nanocomposites are excellent materials for biosensor construction [6, 7]. Amidst the range of materials, polyaniline (PANI) has triggered considerable attention because of its easy fabrication process and low cost. GO exhibits a two-dimensional planar structure with oxygen containing functional groups (carboxyl, carbonyl, hydroxyl, and epoxy) on the basal and edge planes. These highly active functional groups act as binding sites and facilitate the consequent “in situ” polymerization of aniline adhering on the surface and edges of GO planar sheets [8]. In addition, the electrostatic interaction or π stacking between the phenyl group of PANI and the functional moieties on GO also results in “in situ” polymerization on GO surface [9]. Thanks to unusual properties of GO/PANI nanocomposite, such as high conductivity, biocompatibility, redox activity,

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and inexpensive nature, it has often been employed to synthesize composite materials for a wide range of potential applications. Currently, the GO/PANI nanocomposite has attracted considerable attention because of a significant enhancement in the conductivity and amplification of the electrocatalytic activity of substrates [5, 9]. GO-doped PANI anticipates being an excellent material that facilitates a whole range of applications, including biosensors, electrochemical devices, supercapacitors, solar cells, and fuel cells [10].

Adenine (Ade) is an important component of deoxyribonucleic acid (DNA) and is involved in the key function of carrying genetic instructions. Therefore, sensitive determination of Ade indicates relevance in clinical diagnosis. A range of methods have been studied to investigate the Ade concentration [11, 12]. However, thanks to a fast, cost-effective and simple procedure, electrochemical detection has been widely accepted for the investigation of this nucleobase. However, due to the slow electron transfer and weak adsorption on the carbon electrode surface, it exhibits a low sensitivity [13]. The modification of carbon electrodes brings the possibility of overcoming these problems to construct a sensor for the determination of Ade at the respective level. The electrochemical oxidation of Ade has been studied extensively on the glassy carbon electrode modified by different strategies. Nanomaterials and their nanocomposites possess enhanced electrochemical activity, and thus they can be used to modify electrodes. For instance, Gao et al. fabricated porous films of overoxidized polypyrrole/graphene, electrodeposited on the glassy carbon electrode for the detection of adenine and guanine [6]. In addition, Fan et al. demonstrated that TiO₂/graphene nanocomposite possesses excellent electrochemical activity towards electrochemical oxidation of adenine and guanine [14]. In another investigation, Wang et al. reported that the graphene quantum dots carrying an AgNP-GCE were highly effective for simultaneous detection of adenine and guanine [15]. Considering the fascinating properties of graphene-based materials, it is interesting to explore the graphene-based nanocomposites towards the oxidation of Ade. However, to our best knowledge, the electrochemical behavior of Ade using GO/PANI-GCE has not been reported yet. Therefore, the aim of this work is to prepare and characterize the GO/PANI nanocomposite and to study its electrochemical activity towards the Ade oxidation when used as an electrode modifier.

Experimental

Chemicals

All the chemicals used were of analytical grade and purchased from Sigma Aldrich without further purification. GO (catalogue number 763705) dispersed in H₂O at a concentration

of 2 mg/ml, was purchased from Sigma Aldrich (<https://www.sigmaaldrich.com/>). Aniline was purchased from Lach-Ner (<http://www.lach-ner.com/>). MilliQ Direct QUV (Millipore, MA, U.S.A.) (<http://www.emdmillipore.com/>) was used to prepare deionized water.

Preparation of GO/PANI nanocomposite and fabrication of GO/PANI modified GCE

The GO/PANI nanocomposite was prepared as follows: purified aniline (0.3 g, 3.2 mmol.L⁻¹) was dissolved in 10 mL of 1 M HCl aqueous solution. Under continuous vigorous stirring at room temperature, ammonium peroxydisulfate (0.18 g, 0.8 mmol.L⁻¹) was rapidly added into the aniline solution. The characteristic green color of polyaniline (emeraldine salt) appeared after 5 min indicating polymerization of aniline. The solution was allowed to stir at room temperature for 3 h followed by vacuum drying. At the end GO dispersed in water was added to PANI at a concentration of 1 mg.mL⁻¹ at -4 °C, and the resulting GO/PANI nanocomposite was sonicated for 2 h. To prepare a modified electrode, the GCE was polished with 0.05 μm alumina powder and subsequently ultrasonicated in acetone and Milli-Q water for 5 min. The synthesized GO/PANI nanocomposite was placed on a GCE (3 mm diameter) and the electrode surface was dried in air leaving the GCE surface modified.

Electrochemical experiments

Electrochemical signals were recorded using a PGSTAT 101 potentiostat (Metrohm Herisau, Switzerland), and the NOVA 1.8 software (Metrohm Herisau, Switzerland) was employed for data evaluation. An electrochemical robotic device (Sensolytics, Bochum, Germany) performed the automatic positioning of electrodes in the cell. Ag/AgCl/3 M KCl served as a reference electrode (Metrohm, Herisau, Switzerland) and platinum wire (Metrohm, Herisau, Switzerland) as a counter electrode. Glassy carbon electrodes (GCE, CH Instruments, U.S.A.) were used as working electrodes after modification. All electrochemical experiments were conducted at room temperature.

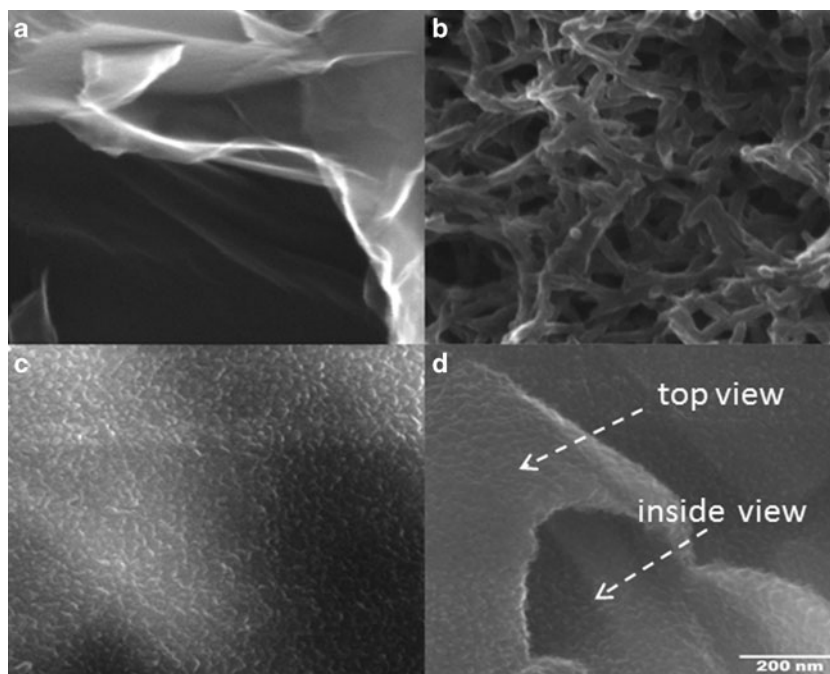
Scanning electron microscopy (SEM)

The morphological characteristics of the purified samples were investigated with a scanning electron microscope (SEM) Mira II LMU from Tescan (Czech Republic). The working distance was 5.01 mm ± 0.1 mm at a voltage of 30 kV.

Raman spectroscopy

The room-temperature Raman spectra of all samples were taken using 10 mW of 633 nm He-Ne excitation and a Renishaw's inVia spectrometer with 1800 l mm⁻¹ grating

Fig. 1 SEM morphology images of (a) graphene oxide, (b) polyaniline, (c) top view, and (d) top-inside view of graphene oxide/polyaniline nanocomposite



and a Peltier cooled CCD detector. All samples were loaded and sealed in glass capillaries. An objective with 20 \times magnification was used for micro-Raman measurements in back-scattering geometry to both excite the sample and collect its Raman response.

UV-VIS absorption spectroscopy

The absorption spectra of GO/PANI nanocomposites were recorded within a range from 200 nm to 900 nm on a SPECORD 210 spectrophotometer (Analytik Jena, Jena, Germany) using UV-transparent cuvettes with 1 cm optical path (Brand, Wertheim, Germany) at 25 $^{\circ}$ C maintained by a Julabo thermostat (Labortechnik, Wasserburg, Germany). The changes in the absorbance spectra of the samples were recorded and evaluated using the WinASPECT program, version 2.2.7.0.

Statistical analysis

All the measurements were performed at least in triplicates. Data were processed by using OriginPro 8.5 software (OriginLab, Northampton, U.S.A.). The limit of detection (LOD) was calculated according to [16].

Results and discussion

Characterization of GO/PANI nanocomposite

GO with tunable reactive oxygen functionalities on basal and edge plane facilitates interaction with other molecules for

synthesizing composites with other materials, including conducting polymers. Conducting polymers such as polyaniline (PANI), polypyrrole (PPy), poly(3,4-ethylenedioxythiophene) (PEDOT), and polythiophene (PTH) have been investigated to achieve enhanced conductivity. Among these polymers, PANI is considered as a promising material because of its cost-effective, easy to prepare, and high capacitive characteristics [17]. We selected GO to make a

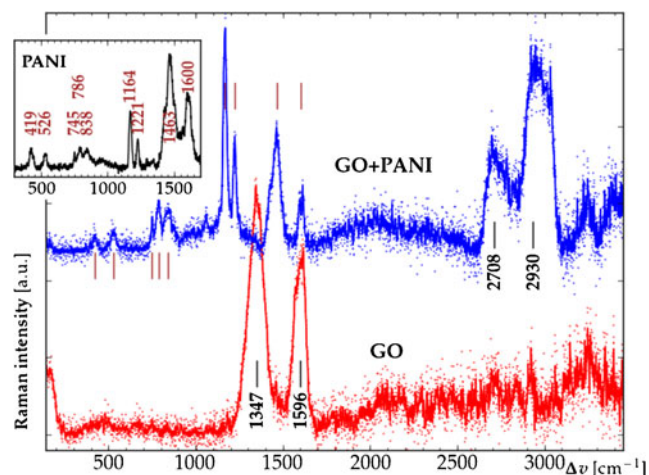


Fig. 2 Raman spectra (points, after baseline subtraction) of graphene oxide and graphene oxide/polyaniline nanocomposite as measured at 1 $\text{mg}\cdot\text{mL}^{-1}$ concentration. The spectra were vertically shifted and moving average (vertical lines) was added for better view. The inset shows a measured spectrum of polyaniline for reference; the positions of its peaks are also indicated in the main figure. Notice the appearance of the second-order carbon peaks due to an interaction of graphene oxide with polyaniline

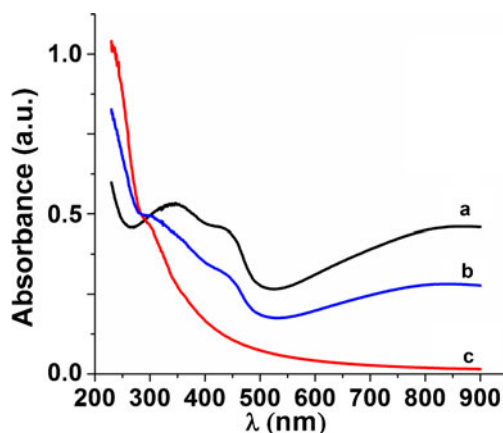


Fig. 3 UV-Vis analysis of (a) graphene oxide/polyaniline nanocomposite, (b) polyaniline, and (c) graphene oxide

composite with PANI because of its many merits such as low cost, stability, simple preparation, and stable dispersion in aqueous solution, which may act as nucleating sites for PANI nanofibers [9, 18]. A homogenous mixture of GO was obtained in water containing polyaniline fibers. The negatively charged graphene oxide facilitates the adsorption of positively charged aniline monomer, which provides a subtle coating of PANI fibers due to the polymerization of aniline on GO sheets. Thus, the probability of adsorption of aniline monomers on GO surface is identical, and it illustrates the formation of a homogenous layer on GO sheets [19]. The morphology of GO, PANI, and the GO/PANI nanocomposite was investigated by SEM (Fig. 1). The 2D morphology of GO sheets can be observed in Fig. 1a.

The drying process probably leads to the formation of GO aggregates, and therefore few packed layers of GO layers can be seen in Fig. 1a. The morphology of PANI can be described as an interconnected branched network of nanowires having a diameter of ~ 50 nm with only few nanometers in length (Fig. 1b). Vertically aligned PANI nanowires on GO can be seen in Fig. 1c. The dense and homogeneous arrangement of PANI nanofibers/wires on GO is a result of GO dispersion in water.

The top-inside investigation by SEM (Fig. 1d) reveals an aligned arrangement of PANI nanowires on GO sheets. Our results are in agreement with another report [20].

To characterize the GO and GO/PANI nanocomposite, Raman spectra were recorded at $1 \text{ mg}\cdot\text{mL}^{-1}$ concentration of GO and GO/PANI nanocomposites, and the resulting spectrum is presented in Fig. 2. The spectrum of GO displays two prominent peaks at 1347 and 1596 cm^{-1} , corresponding to the D-band and G-band respectively, in agreement with the literature [21]. For pure PANI and GO/PANI samples, the peaks indexed at 419 , 526 , 786 , 1164 , 1221 , 1463 , and 1600 cm^{-1} are assigned to the in-plane C–H bending of the quinoid ring, the in-plane C–H bending of the benzenoid ring, C–N⁺ stretching, C=N stretching of quinoid, C=C stretching vibration of the quinoid ring, and C=C stretching of the benzenoid ring respectively, thus indicating the presence of a doped PANI structure [22–24].

The PANI peak positions coincide with the spectral features in the GO/PANI composite, thus suggesting that PANI remains intact after reaction. The disappearance of the D and G carbon peaks at 1347 and 1596 cm^{-1} after the interaction of GO with PANI indicates a successful formation of nanocomposite, thus suggesting intercalation of PANI via π stacking between graphene oxide sheets.

Furthermore, Fig. 3 displayed UV characterization of the GO/PANI nanocomposite; besides the absorption bands of GO at around 300 nm [25], the absorption band at 450 nm corresponds to PANI [26]. In the GO/PANI nanocomposite the absorption band of GO shifted towards the IR region at around 350 nm regardless of the absorption band of PANI, which suggests a highly doped PANI nanocomposite [9].

Electrochemical behavior of adenine on GO/PANI-GCE

The first electrochemical investigation of Ade on a GO/PANI modified GC electrode was performed using cyclic voltammetry in a potential range from 0 to 1.6 V (vs. Ag/AgCl/3MKCl). Figure 4a shows well-defined redox peaks originated from the layer of GO, PANI, and GO/PANI on GCE. The peaks can be attributed to aniline and oxygenated functional

Fig. 4 Cyclic voltammograms of (a) 0.1 M phosphate buffer pH 4 and (b) 0.1 mM adenine in 0.1 M phosphate buffer pH 4 at a bare, graphene oxide (GO), polyaniline (PANI), and graphene oxide/polyaniline (GO/PANI) modified GCE; scan rate 50 mVs^{-1}

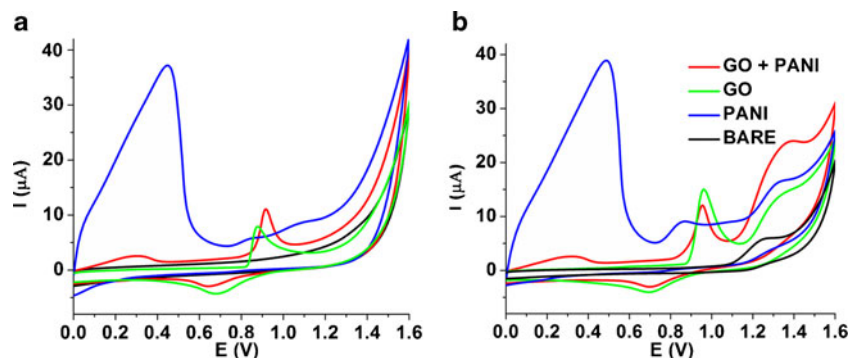
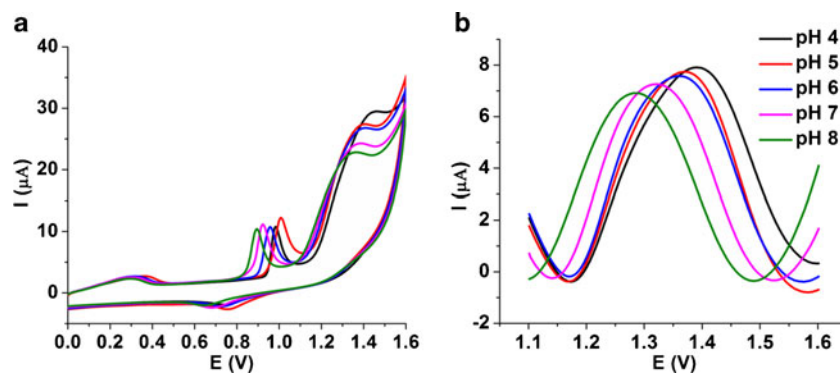


Fig. 5 Cyclic voltammograms of (a) adenine and (b) adenine oxidation peaks after baseline correction as a function of pH on graphene oxide/polyaniline modified electrode in 0.1 M phosphate buffer; scan rate 50 mVs^{-1}



moieties on the surface of GO [17]. It may be noted that the form of interaction of PANI and GO may affect the shape and the potential position of cyclic voltammograms. The decrease of the oxidation peak of PANI in the GO/PANI nanocomposite is likely due to the interaction of GO and PANI. Also, PANI is oxidized while GO is reduced to graphene, as can be seen in Fig. 4a. The oxidation peak currents at potentials around 0.25 V and 0.95 V can be assigned to PANI and GO, whereas the cathodic current peak at around 0.65 V is caused by the reduction of GO as shown in Fig. 4a. GO/PANI nanocomposites showed an improved conductivity of 10 S cm^{-1} and a specific capacitance of 531 F g^{-1} compared to 2 S cm^{-1} and a specific capacitance of 216 F g^{-1} of pure PANI [27] and theoretical high specific surface area of GO ($2630 \text{ m}^2 \text{ g}^{-1}$) [28] contribute to an increase in the electrochemical signals. Electrical conductivity of GO ($0.53 \pm 0.39 \times 10^{-3} \text{ S m}^{-1}$) is by orders of magnitude lower than the pristine graphene (35100 S m^{-1}) due to the presence of free oxygenated groups. However, these oxygenated groups make the surface reactive and appropriate for composite formation through chemical or π interactions [29, 30]. This is in good agreement with the results shown in Fig. 4b and Fig. S1. The electrochemical oxidation of Ade on GO was comparatively lower than PANI, perhaps due to the non-conductive nature of GO, whereas the electrochemical oxidation of Ade on GO/PANI modified GCE exhibits a remarkable enhancement in current density relative to the GO, PANI, and bare electrode (Fig. S1). The adsorption mechanism of Ade on modified electrode is hard to confirm but the adsorption is confirmed by the effect

of scan rate. However, adenine forms probably a complex through π transitions with oxygenated chemical functional groups on the GO/PANI and influences the interaction between Ade and modified electrode surface. It has been reported that this purine adsorbed in a variety of conformations on substrates due to its different interaction sites [31]. Well defined oxidation peak of Ade on modified electrode ($24.4 \mu\text{A}$) observed at potential 1.37 V, compared to bare electrode ($5.2 \mu\text{A}$) at a potential of 1.26 V, indicates a shift of $\sim 0.1 \text{ V}$ towards the positive potential. It is convenient to say that the 5 fold increase was obtained thanks to the modification.

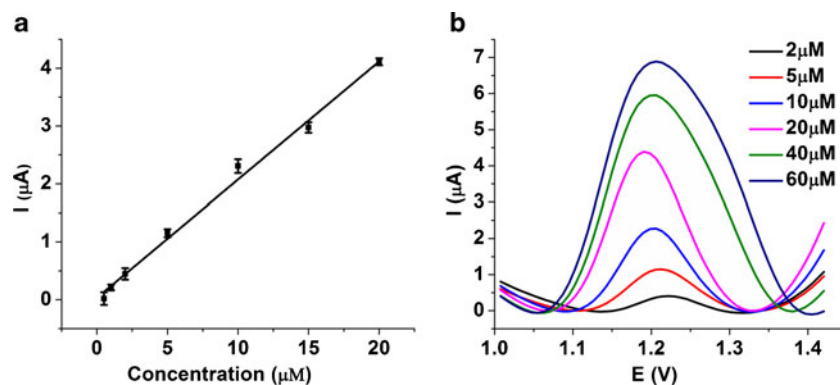
Effect of scan rate

The effect of the scan rate on electrochemical response of 0.1 mM Ade in 0.1 M phosphate buffer (pH 4.0) on a GO/PANI modified electrode was investigated by cyclic voltammetry. Fig. S2 depicts the oxidation peak current of Ade increased linearly corresponding to the scan rate in a range of $25\text{--}400 \text{ mVs}^{-1}$. The linear regression relation between the oxidation current and the scan rates can be expressed as $I_{\text{pa}} (\mu\text{A}) = 0.712 + 5.53 \nu/\text{mVs}^{-1}$ ($R^2 = 0.9880$) ($n = 5$), thus indicating that the adsorption of Ade was a surface-controlled process rather than a diffusion process on the electrode surface. Furthermore, from the slope of the linear plot of I_{a} vs ν , the surface concentration of Ade can be assessed to approximate $2.1 \times 10^{-10} \text{ mol cm}^{-2}$, according to the published information [32]. The dependence of the voltammetric peak height on scan rate can be utilized successfully by the

Table 1 Data from the cyclic voltammetry measurements of 0.1 mM adenine, at different pH (from 4 to 8); scan rate 50 mVs^{-1}

pH	Bare			GO/PANI		
	$I_{\text{pa}} (\mu\text{A})$	$E_{\text{pa}} (\text{V})$	Charge density ($\mu\text{A}\cdot\text{cm}^{-2}$)	$I_{\text{pa}} (\mu\text{A})$	$E_{\text{pa}} (\text{V})$	Charge density ($\mu\text{A}\cdot\text{cm}^{-2}$)
4	1.70	1.280	5.7×10^{-4}	7.98	1.386	2.64×10^{-3}
5	1.56	1.181	5.2×10^{-4}	7.74	1.350	2.58×10^{-3}
6	1.35	1.10	4.50×10^{-4}	7.45	1.329	2.49×10^{-3}
7	1.21	1.01	4.03×10^{-4}	7.04	1.3	2.35×10^{-3}
8	0.98	0.939	3.27×10^{-4}	6.62	1.28	2.21×10^{-3}

Fig. 6 Adenine determination by DPV measurements on graphene oxide/polyaniline modified GCE in 0.1 M phosphate buffer pH 4; adenine oxidation peak was observed around 1.2 V (vs *Ag/AgCl*): (a) calibration plot of adenine at 0.5–20 μM concentration; (b) adenine oxidation peak at 2–60 μM concentration



voltammetric elimination procedure (EVP) for the evaluation of actual processes at the electrode surface. The oxidation peaks at potentials around 0.25 V and 0.95 V, attributed to PANI, were also investigated by EVP. While the elimination signals of the second oxidation process (at about 1 V) are 7–8 times higher than the original LSV oxidation signals, the first oxidation EVP signals (at about 0.3 V) show surprisingly negative current values (Fig. S3). The shape of pre-peaks and counter-peaks of the second EVP signal indicates a significant participation of GO/PANI morphology in the process of electron and proton transfer.

Effect of pH on the electrochemical oxidation of adenine on modified electrode

The effect of pH on the electrochemical behavior of Ade was investigated. Phosphate buffer (0.1 M) was selected as a supporting electrolyte with pH ranging from 4 to 8. Cyclic voltammetry was performed with 0.1 mM Ade at a scan rate of 50 mVs^{-1} (Fig. 5).

An expected increase of the oxidation peak current and simultaneously an anodic shift was observed as a function of pH, which can be explained as a consequence of protonation of Ade, with a pKa value of 4.1. The electro-oxidation of Ade shifts towards positive potential with decreasing pH value due to the mono-protonation of Ade, which leads to an unequal charge distribution in the Ade ring, due to which the chemical interaction between Ade and GO/PANI nanocomposites is influenced. The process noticed was irreversible as no reduction peak of Ade was observed in cyclic voltammograms. The parameters derived from the experiments are given in Table 1.

The linear dependence (Fig. S4a) between the peak current and pH on the modified electrode was found to be $I_{\text{pa}} (\mu\text{A}) = -0.3808 \text{ pH} + 9.2680$, $R^2 = 0.9857$, whereas the linear regression (Fig. S4b) between the peak potential and pH was found to be $E_{\text{pa}} (\text{V}) = -0.025 \text{ pH} + 1.4835$, $R^2 = 0.987$. The enhanced oxidation peak current at modified GO/PANI electrode suggests that the bare electrode is less sensitive towards the Ade oxidation process. The two different oxidation processes of Ade were observed on bare and modified electrodes (Fig. S4b), which can be attributed to the nanocomposite modification. According to $\frac{dE_p}{d(\text{pH})} = -\frac{0.059}{\alpha n_a} p$ (α is the electron transfer coefficient, n_a is the number of electrons involved in the potential controlling reaction, p is the number of protons) it should be noted that for a GO/PANI modified electrode the ratio of the number of protons to the number of electrons is about half. The slope of E_p vs. pH is 0.025 V/pH on GO/PANI modified electrode indicates an unequal number of protons corresponding to the number of electrons in the rate determining step. By contrast, it is not in agreement with the available literature [33, 34].

Quantification of adenine on modified electrode

The determination of Ade was investigated by differential pulse voltammetry (DPV). Figure 6 shows the linear response of a GO/PANI modified GCE electrode for various concentrations of Ade. The oxidation peak current increases linearly within the range of 0.5–20 μM concentration of Ade. The calibration curve of Ade, shown in

Table 2 A comparison of analytical parameters of different chemically modified electrodes for the investigation of adenine

Electrode	Modification	Linear range (μM)	Detection limit (μM)	Reference
GCE	PANI/MnO ₂	10–100	2.9	[34]
GCE	TiO ₂ /Gr	0.5–200	0.1	[14]
GCE	Gr/IL/CS	1.5–350	0.5	[35]
GCE	PANI/GO	0.5–20	0.07	This work

Fig. 6a, exhibits a linear regression equation I_{pa} (μA) = 0.034 + 0.204 c (μM) ($R^2 = 0.996$).

The limit of detection was calculated by using IUPAC (International Union of Pure and Applied Chemistry) norms [16]. The results demonstrated sensitivity of the analytical performance for detection of Ade with a detection limit (LOD) of 72 nM and a corresponding limit of quantification (LOQ) of 240 nM. A comparison of GO/PANI nanocomposite-modified GCE with other surface modification methods used for Ade detection shown in the literature is in Table 2. However, the system proposed shows a limitation towards the electrochemical sensing of AMP, ADP, ATP, guanine, cytosine, and thymine. These molecules were investigated at a concentration of 0.1 mM and no signal was observed.

Furthermore, the reproducibility and stability of the GO/PANI modified GCE was investigated by the differential pulse voltammetry of 0.1 mM Ade in 0.1 M phosphate buffer pH 4.0. The relative standard deviation (RSD) of the oxidation peak current of Ade in determinations on individual modified electrodes was observed approximately in 4.7 %, which indicates a good reproducibility of the electrode. The stability of the prepared nanocomposite material was tested according to the recorded peak current values in a two-week period. During this period no relevant decrease (higher than RSD, $n = 5$) of the peak currents obtained was observed.

Application of GO/PANI modified electrode in serum samples

Under the optimum conditions (pH 4.0, 50 mV/s) the proposed modification was further validated for selectivity in the media including the different concentration of human serum samples. The determination of Ade (0.1 mM) in the presence of other biological ions/molecules represented by human serum was investigated. The results indicated that the sensor demonstrates good selectivity for Ade. However, increased serum concentrations from 0.05 mM to 0.3 mM interfere significantly in the detection of Ade (Fig. S5). The reliability and sensitivity of this electrode provides promising results for the use of this technique to detect Ade in the biological matrix.

Conclusions

Combining the advantageous features of GO and PANI, the GO/PANI nanocomposite was prepared by conventional chemical oxidative polymerization of aniline by ammonium peroxydisulfate, and used for the modification of GCE, which shows a promising activity towards the detection of Ade. The sensor proposed exhibited good reproducibility, sensitivity and stability towards adenine detection. Moreover, the method proposed was used for significant detection of adenine at

micromolar concentration with an increased concentration of human serum samples with high accuracy. However, the GO/PANI modified electrode showed a limitation towards the sensing abilities of AMP, ADP, ATP, guanine (overlapping with material peak), cytosine, and thymine (limited potential window). GCE modified with GO/PANI nanocomposite brings a new possibility for the detection of Ade in bioanalytical laboratories in medicine and pharmacy.

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Compliance with Ethical Standards The author(s) declare that they have no competing interests.

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