

Reduced Graphene Oxide/ α -Cyclodextrin-Based Electrochemical Sensor: Characterization and Simultaneous Detection of Adenine, Guanine and Thymine

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Abstract: Graphene, the rising star of carbon nanomaterials, is a single layer of sp^2 -bonded carbon atoms patterned in a 2D honeycomb network. Thanks to its unique features, graphene has attracted enormous attention and it has arisen various applications in the fields of optical and electrochemical sensors. In the present work, reduced graphene oxide/ α -cyclodextrin (*rGO*/ α -*CD*) is proposed as a nanocomposite for individual and simultaneous detection of adenine, guanine and thymine. *rGO*/ α -*CD* has been characterized by FT-IR, Raman spectroscopy, AFM, HR-TEM and SEM techniques. Cyclic voltammetry, differential pulse voltammetry and chronoamperometry techniques were utilized for detection of adenine, guanine and thymine. The limit of detection (LOD) values for adenine, guanine and thymine were calculated to be 145.5, 38.9 and 52.9 nmol L⁻¹, respectively. The results show that the developed sensor can be utilized for the determination of adenine, guanine and thymine in human serum, indicating its promising application in the analysis of real samples.

İndirgenmiş Grafen Oksit/ α -Siklodekstrin Esaslı Elektrokimyasal Sensör: Karakterizasyon ve Adenin, Guanin ve Timinin Eşzamanlı Tayini

Anahtar Kelimeler

Grafen oksit,
Siklodekstrin,
Elektrokimyasal sensör,
Adenin,
Guanin,
Timin

Özet: Karbon nano materyallerin yükselen yıldızı olan grafen, sp^2 -hibritleşmesi yapmış ve iki boyutlu bal peteği şeklinde düzenlenmiş olan karbon atomlarının bir atom kalınlığında oluşturduğu tabakadır. Benzersiz özellikleri sayesinde, grafen büyük ilgi uyandırmış olup optik ve elektrokimyasal sensörler alanında çok çeşitli uygulama alanlarının gelişmesine katkı sağlamıştır. Bu çalışmada, adenin, guanin ve timinin tek başına ve eş zamanlı tayinlerini gerçekleştirebilmek için indirgenmiş grafen oksit/alfa siklodekstrin (*rGO*/ α -*CD*) nano kompozit malzemesi önerilmektedir. *rGO*/ α -*CD* materyali FT-IR, Raman spektroskopisi, AFM, HR-TEM ve SEM teknikleri ile karakterize edilmiştir. Adenin, guanin ve timin tayini için dönüşümlü voltametri, diferansiyel puls voltametri ve kronoamperometri teknikleri kullanılmıştır. Adenin, guanin ve timin için en düşük tayin limitleri 145.5, 38.9 ve 52.9 nmol L⁻¹ olarak hesaplanmıştır. Çalışma sonuçları, önerilen bu sensörün adenin, guanin ve timinin insan serumu içerisinde tayin edilebileceğini ve dolayısıyla gerçek numune analizinde umut vadeden bir uygulama olduğunu göstermiştir.

1. Introduction

Graphene, defined as miracle material of the future, is a very thin and strong carbon-based nanomaterial that shows great promise in various applications owing to its large surface area, excellent electron transfer performance, high mechanical/chemical strength and pretty high thermal conductivity features [1–3]. With its unique features, graphene is considered as an excellent supporting material and it

has attracted a great attention of researchers in both academic and industrial fields [2,4]. In recent years, a great interest has grown for investigation of graphene and related materials having various new characteristics by either covalent/non-covalent interactions of metal nanoparticles, polymers or toroidal-shaped macromolecules such as cyclodextrins [5–8]. Cyclodextrins (CDs) are oligosaccharides (α -, β -, or γ -CD) with a lipophilic inner cavity and a hydrophilic outer surface

[9]. They are extensively used in electrochemical sensor area due to this interesting characteristics enabling to form stable complexes with various kind of analytes such as organic, inorganic compounds and biological molecules [10–13]. The combination of unique features of graphene and cyclodextrins gives rise to synergetic effects for being applied in various fields of sensor area, particularly in electrochemical sensors. Recently, Zhang et al. have proposed a convenient sensor by using β -cyclodextrin-graphene for the electrochemical determination of paichongding [14]. Jiang et al. have reported electrochemical sensing of gatifloxacin by utilizing electro-polymerized β -CD and rGO composite [15]. Zhan et al. have used β -CD-rGO nanocomposite for electrochemical detection of Pb^{2+} [16]. Subsequently, these kind of nanocomposites have been utilized by modification of electrode surfaces for detection of specific analytes.

Deoxyribonucleic acid (DNA), which is the most important biological macromolecule, is well-suited for biological information storage [13]. Adenine, guanine, cytosine and thymine are the fundamental purine bases existed in DNA [17]. Due to the fact that the abnormal alterations in their concentration in living organisms designate the efficiency of the immune system and may cause various diseases, the determination of their individual concentrations or ratio in DNA has crucial role for the determination of nucleic acid quantity [18]. Up to now, considerable attention has been expended on the development of biosensors for detection of these purine bases. In this respect, electrochemical biosensors show excellent selectivity and sensitivity because the analyte can be readily identified by its current and/or peak potential change [19].

Given the aforementioned context, herein, a graphene-based electrochemical sensor (*rGO/ α -CD/GCE*) is proposed for individual and simultaneous detection of adenine, guanine and thymine. This paper is the first report dealing with the design and construction of *rGO/ α -CD* composite for the simultaneous determination of adenine, guanine and thymine. In addition, the proposed sensor enables the detection of these purine bases in human serum, which indicates its applicability as a promising platform in the analysis of real samples.

2. Material and Method

2.1. Chemicals and equipments

All chemicals were obtained from Global suppliers and used considering the material safety data sheet. Human serum was not collected from human, it was purchased from Sigma-Aldrich (H4522) and used by 10 fold dilution. All aqueous solutions were freshly prepared using pure-water obtained by Milli-Q system of Millipore.

A Perkin Elmer 100 FT-IR was used for Fourier transformed infrared (FT-IR) spectra of the samples. A Renishaw inVia Reflex (Gloucestershire, U.K.) equipped with CCD detector and a Leica microscope was acquired to record Raman spectra. A ZEISS EVO LS 10 SEM was performed for SEM images. AFM images were acquired using a Park XE7 instrument. A JEOL JEM 2100F HR-TEM was used for TEM images.

Electrochemical experiments were performed in a three-electrode cell containing phosphate buffer solution (PBS) with 0.10 M KCl at pH 7.4. GCE was used as working electrode. A platinum wire and Ag/AgCl electrode were respectively used as reference electrode and counter electrode. The cell was powered by IVIUM-CompactStat potentiostat.

2.2. The production of α -Cyclodextrin decorated graphene (*rGO/ α -CD*) nanocomposite

Aiming at producing *rGO/ α -CD* nanosheets, graphene oxide (*GO*) was initially produced by following modified Hummers' approach with an additional pre-oxidation process. Then, *rGO/ α -CD* nanosheets were obtained by following the previously reported procedure [5]. Briefly, a 40.0 mL of *GO* dispersion (0.5 mg mL^{-1}) was sonicated for 1 h. After that, 40.0 mL of 160 mg mL^{-1} of α -CD aqueous solution and $600.0 \text{ }\mu\text{L}$ of ammonia solution were transferred to the *GO* solution. After pouring of $40 \text{ }\mu\text{L}$ of hydrazine, the mixture was shaken for 5 minutes. The resultant was stirred at $60 \text{ }^\circ\text{C}$ for 4 h. The black dispersion was filtered using $0.22 \text{ }\mu\text{m}$ pore-sized membrane filter to obtain *rGO/ α -CD* nanosheets.

2.3. Procedure for electrochemical measurements

Electrochemical experiments were performed at $25 \text{ }^\circ\text{C}$. For modification of composite, GCE surface was polished with alumina dispersion on a felt-pad. Afterwards, GCE was rinsed with pure water and respectively sonicated in water and methanol for 20 min. After drying at room temperature, $5.0 \text{ }\mu\text{L}$ of composite (0.2 mg/mL) was casted on it. Lastly, *rGO/ α -CD/GCE*, used as sensor, was dried prior to use.

3. Results

3.1. Characterization of nanocomposite

The structural evaluation of the nanocomposite was performed using FT-IR and Raman spectroscopy (see Figure 1) and compared to *GO* and *rGO*. The first evidence of the successful functionalization of graphene oxide sheets was evaluated by FT-IR. Figure 1A shows that *GO* and *rGO/ α -CD* samples possess hydrophilic O–H group ($3010\text{--}3650 \text{ cm}^{-1}$) on their surfaces, thus ensuring their good dispersibility in water [10]. A stretching vibration of CH_2 between 2830 and 2950 cm^{-1} was obtained in the spectra.

Furthermore, aromatic C=C stretching modes at 1630, 1671 and 1642 cm^{-1} belongs to GO, rGO and rGO/ α -CD, respectively [5]. The characteristic stretching vibrational modes of C=O (1731 cm^{-1}), C–O (1219 cm^{-1}) and C–O (1051 cm^{-1}) bonds were observed in GO spectrum. This comparison for GO, rGO and rGO/ α -CD explains two important observations. The first is both the disappearance of the broad O–H band by reducing GO into rGO and an enhancement in the case of rGO/ α -CD was observed due to hydroxyl groups on α -CD scaffold, enhancing hydrophilicity of rGO/ α -CD. The second is the existence of characteristic α -CD absorption bands (1153 and 1029 cm^{-1}) depicting the settlement of α -CD on rGO.

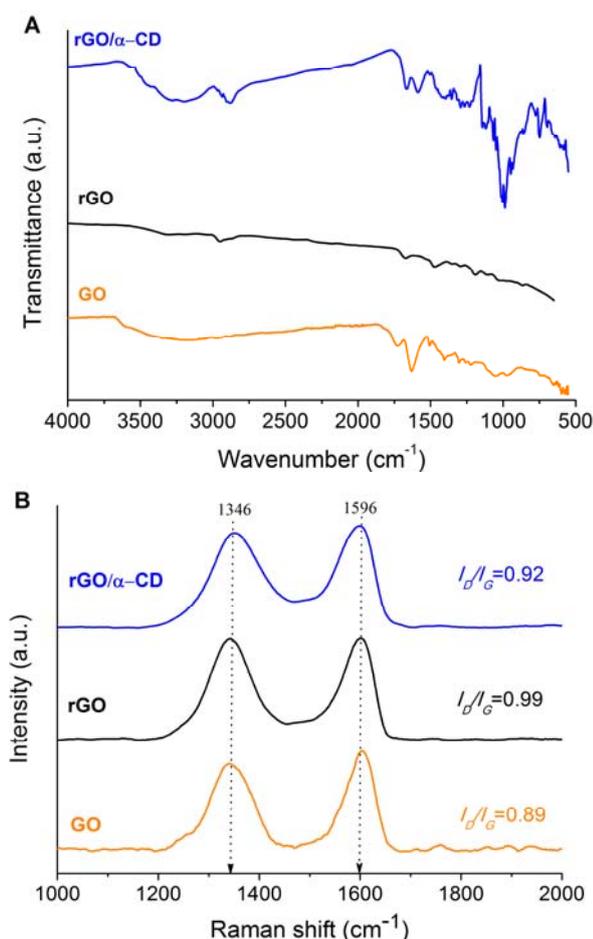


Figure 1. FT-IR (A) and Raman spectra (B) of GO, rGO and rGO/ α -CD composite.

Raman spectroscopy analysis was performed to examine the structural changes after functionalization process. Figure 1B shows the characteristics D and G bands of GO, rGO and rGO/ α -CD at 1346 cm^{-1} and 1596 cm^{-1} . These bands are respectively associated with the sp^3 disorders in hexagonal lattice caused by oxidation/reduction and the vibration of sp^2 -bonded carbon atoms in the graphitic lattice [20,21]. The I_D/I_G ratios were evaluated to be 0.89, 0.99 and 0.92 for GO, rGO and rGO/ α -CD, respectively. These values clearly express that the reduction process leads to disorders in

lattice but settlement of α -CD prevents some functional groups on hexagonal scaffold of graphene layers [5].

Figure 2 shows AFM, TEM and SEM micrographs of GO, rGO and rGO/ α -CD. GO displays its soft nature with the wrinkles and overlaps [22], see Figure 2A/D/G. Figure 2B/E/H show the images of rGO revealing that graphene layers are agglomerated due the removal of oxygen-containing groups leading to hydrophobic forces [23]. Figure 2C/F/I show the AFM, TEM and SEM micrographs of rGO/ α -CD in which, alike GO, a wrinkled topology was clearly observed [5].

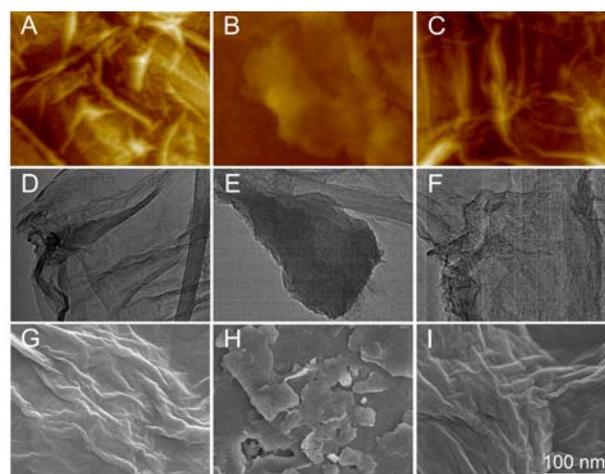


Figure 2. AFM (A-C), TEM (D-F) and SEM (G-I) images of GO, rGO and rGO/ α -CD.

3.2. Electrochemical detection studies

Aiming at investigating the electrochemical detection of adenine, guanine and thymine, cyclic and differential pulse voltammetry techniques were performed. Figure 3 shows the individual differential pulse voltammograms (DPVs) and cyclic voltammograms (CVs, insets of figures) of adenine (Figure 3A), guanine (Figure 3B) and thymine (Figure 3C) in PBS solution (pH 7.4). As obviously seen, guanine, adenine and thymine exhibit characteristic oxidation peaks at 0.67 V, 1.05 V, 1.17 V, respectively. It should be remarked that the enhancement for concentration of adenine, guanine and thymine leads to a linear increase at the oxidation peak currents for three compounds.

3.3. Simultaneous detection of adenine, guanine and thymine

Figure 4A shows DPVs of adenine, guanine and thymine between the concentration range of 10 μM and 50 μM . It is obviously seen that a linear increase is observed in current intensity as a function of concentration. Aiming at evaluating the performance of this sensor for individual determination of adenine, guanine or thymine, DPVs were recorded in the presence of constant quantity of two of them by

changing concentration of the third one Figure 4B-D. A well-defined increase in the intensity of oxidation peak current for the tested analyte was observed (Figure 4E), whereas the current intensity of the others remained stable depicting no interference effect would be existed for each other. These results clearly depict that the three purine bases can be detected in a mixture at the concentration range examined. Herein, the limit of detection (LOD) values for adenine, guanine and thymine was respectively calculated to be, 145.5, 38.9 and 52.9 nmol L⁻¹ by using the standard deviation of y-intercept and the slope of the regression line ($LOD=3S_a/b$, where S_a is the standard deviation of the response and b is the slope of the calibration curve) [24,25].

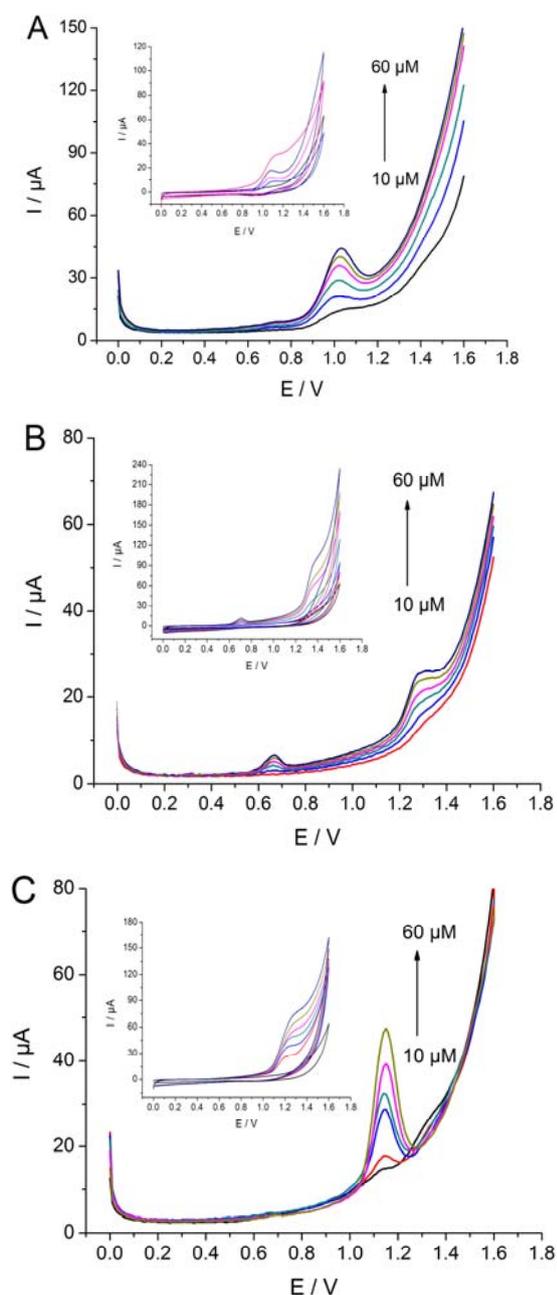


Figure 3. DPVs of adenine (A), guanine (B) and thymine (C). Pulse amplitude is 0.05 V. The insets CVs of the corresponding analytes. Scan rate is 0.1 V s⁻¹ for CV.

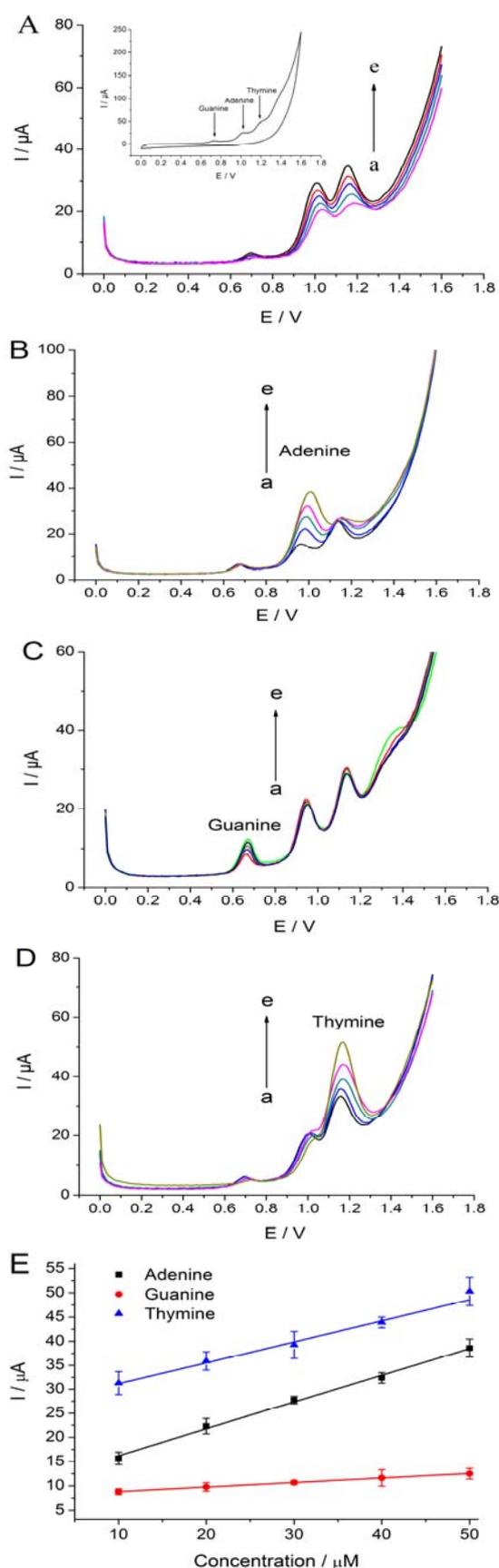


Figure 4. DPVs in the presence of three analyte for (a) 10, (b) 20, (c) 30, (d) 40, (e) 50 μ M (A). The inset shows the anodic CV for the presence of three analytes (50 μ M). Scan rate is 0.1 V s⁻¹. DPVs of adenine (B), guanine (C) and thymine (D). Pulse amplitude was 0.05 V. Linear change for current intensity as a function of analyte concentration (E).

Finally the reproducibility of the proposed sensor was investigated by successive detection of 10 μM adenine, guanine and thymine with five independently prepared electrodes, and the relative standard deviations (RSD) was calculated to be 3.6%, 2.1% and 4.7% respectively, thus demonstrating good reproducibility. The repeatability of the proposed sensor was evaluated by examining the voltammetric responses five times, and the RSD was calculated to be 5.1%, 4.3%, and 6.2% for adenine, guanine and thymine, respectively. The stability of the modified electrode was tested by detection of 10 μM adenine, guanine and thymine after storage for 2 weeks and no obvious decline was observed in the oxidation peak currents response with the RSDs of 4.9%, 4.6%, and 5.4% toward adenine, guanine and thymine, respectively, which could be ascribed to the long-term stability.

3.4. Real sample applications

Aiming at performing the modified electrode for biological samples, adenine, guanine and thymine solutions was spiked in the human serum sample in the presence of various interferent agents, ascorbic acid (AA), uric acid (UA) and glucose (Glu). The interference effects of 0.1 AA, 0.1 mM UA and 0.1 mM Glu on the chronoamperometric response of 10 μM adenine, guanine and thymine were tested (Figure 5). No change in current intensity of adenine, guanine and thymine were observed in the presence of related interferents. In the mixture of all compounds, the addition of target analyte (adenine, guanine and thymine) resulted in a considerable increase at current intensity. Thus, it should be remarked that the proposed sensor can be successfully utilized for the detection of adenine, guanine and thymine for the clinical application in the presence of the related interferent agents.

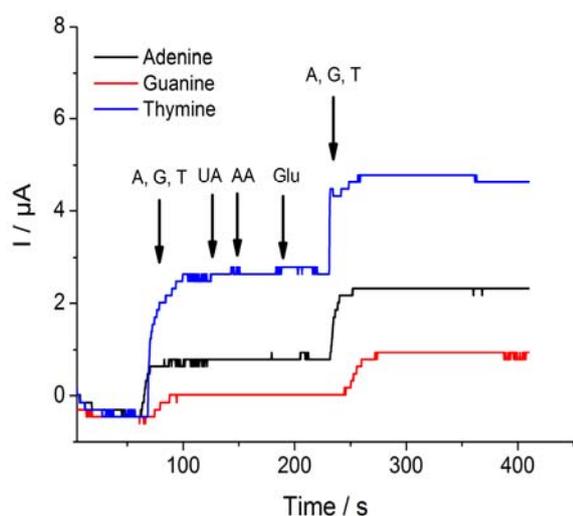


Figure 5. Chronoamperometric responses of $rGO/\alpha\text{-CD}/GCE$ and interference effect of foreign species (0.1 mM of UA, AA, Glu) on the determination of adenine, guanine and thymine (10 μM of each analyte) in the 10-fold diluted human serum in PBS solution (pH 7.4).

4. Discussion and Conclusion

A $rGO/\alpha\text{-CD}$ modified glassy carbon electrode was prepared and used for individual and simultaneous detection of adenine, guanine and thymine as voltammetric sensor. The basic mechanism is based on interaction of $\alpha\text{-CD}$ with analyte. Herein, $\alpha\text{-CD}$ ensures both more accessible active sites to capture analyte and rGO accelerates electron transfer leading $rGO/\alpha\text{-CD}$ to show high electrochemical activity. In the literature, $\beta\text{-CD}$ combined with graphene is widely studied [5,10,16] and there are some reports for detection of nucleobases [13]. In addition, $\gamma\text{-CD}$ has a larger cavity which may not be suitable for selective detection of adenine, guanine, thymine. However, $\alpha\text{-CD}$ has a smaller cavity and not largely used in selective detection of biologically important analytes. Thus, $\alpha\text{-CD}$ was chosen for this study to gain novelty for the literature and to show availability of $\alpha\text{-CD}$ for the sensor applications.

Apart from individual determination, simultaneous determination was successfully achieved. The peak currents exhibited favorable linear relationship as a function of analyte concentration. Considering the linear change, LOD values were calculated and compared to the previously published papers [13,17,26,27]. The comparison for LOD values are tabulated in Table 1. As easily seen that LOD values are in some papers are higher than the calculated in this paper which indicates that the proposed sensor displays a good performance for the tested analytes. Moreover, in the some published papers, only two analytes were examined whereas three analytes were investigated in this work. The detection of cytosine could not be achieved because an oxidation peak occurs for guanine at the same characteristic peak potential of cytosine (around +1.4 V). In addition, cytosine peak appears around the limit of potential window at high concentrations whereas almost no response was obtained for the cytosine in the experimented concentrations for the other tested analytes (from 10 to 60 μM).

Table 1. Comparison of LOD values of various samples for determination of purine bases.

Sample	Analyte	LOD (nmol L^{-1})	Reference
PANI/ MnO_2	A	7700	[17]
	G	7800	
	T	4900	
GCE/GMC	A	630	[26]
	G	760	
Graphene-NF	A	900	[27]
	G	1900	
AgNPs- $\beta\text{-CD}$ -Gr	A	150	[13]
	G	90	
	T	90	
$rGO/\alpha\text{-CD}$	A	145.5	This study
	G	38.9	
	T	52.9	

Apart from individual and simultaneous determination, the effect of interferents was investigated and the results showed that the

proposed sensor could be successfully performed in the evaluation of adenine, guanine and thymine consisting of AA, UA and Glu. From the results aforementioned above, it can be concluded that the proposed simple sensor can be used as a convenient sensing probe for detection of adenine, guanine and thymine, which can be a promising model for real samples analysis.

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