Fe₂V₄O₁₃ Nanoparticles Based Electrochemical Sensor for the Simultaneous Determination of Guanine and Adenine at Nanomolar Concentration

Prashanth Shivappa Adarakatti, Mallappa Mahanthappa, Eranjaneya H, and Ashoka Siddaramanna

Abstract: A simple strategy has been proposed for the simultaneous quantification of guanine (GU) and adenine (AD) using Fe₂V₄O₁₃ nanoparticles (Fe₂V₄O₁₃ NPs) modified carbon paste electrode (Fe₂V₄O₁₃ NPs/CPE) in phosphate buffer solution (PBS). The Fe₂V₄O₁₃ NPs were prepared by a simple solution combustion method where sucrose was used as a fuel. The electrochemical behavior of GU and AD at the electrochemical interface has been studied by using cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPSV). The results illustrate that the Fe₂V₄O₁₃ NPs shows enhanced electrocatalytic activity and voltammetric response towards GU and AD. The proposed sensor showed linearity between the concentration 0.5 and 60 µM with limit of detection (LOD) 32 and 37 nM for GU and AD respectively. The sensitivity towards GU and AD were respectively found to be 1.393 and 1.851 µA/µM. Further, the proposed electrochemical sensor has been successfully employed to determine GU and AD contents in milk powder and calf thymus DNA samples.

Keywords: Fe₂V₄O₁₃ nanoparticles · Purine bases · Carbon paste electrode · Electrochemical sensor

1 Introduction

Biomolecules have gained much attention in the area of biological field and considered as major cellular target to responsible many physiological function. Deoxyribonucleic acid (DNA) is an potentially active biomolecule which plays an vital role in the gene transcription, mutagenesis and gene expression, and thus can be portrayed as one of the nature’s most elementary conduits for the development and functioning of living organisms [1]. Adenine and guanine are important bioanalytes and involved in the construction of DNA, RNA and other biologically significant species [2]. Abnormal concentration of these biomolecules affects the activities of catabolic, anabolic, inter conversion of enzymes [3] and thereby causes various diseases like Alzheimer’s, diabetes, Parkinson’s, cancer, macular degeneration and HIV/AIDS [4]. Thus, the determination of these biomolecules is very crucial in the clinical diagnosis and pharmaceutical formulation. Considering this aspect, simple and rapid with highly sensitive and selective detection methodology is essential.

The certified analytical approaches in practice for biomolecules are spectrophotometry, capillary electrophoresis and liquid chromatography [5–7]. Though these methods are exceptionally good, required trained personnel and not suitable for routine analysis. In order to overcome these limitations, electrochemical techniques made their signature within the electroanalysis domain as an important tool for the detection of biomolecules [8–11], where simultaneous detection of adenine and guanine using unmodified carbon paste/glassy carbon electrode is difficult owing to their overlapping of their oxidation peaks and sluggish electrochemical oxidation reaction.

Mercury based electrodes have been extensively used in the past for biomolecule detection due to its better sensitivities, but its usage has been stopped worldwide due to its high toxic profile [12]. Chemically modified electrodes (CMEs) have gained much interest in recent years owing to their outstanding selectivity and specificity towards target analytes [13–17]. Several chemically modified electrochemical sensors have been reported for monitoring of biomolecules by stripping mode, because the stripping techniques provide better detection limits than conventional methods [18,19]. Numerous reports have been appeared recently on the application of various forms of substrate materials for the determination of GU

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and AD using stripping mode. For instance, TiO₂/graphene nanocomposite/GCE [20], graphene-nafion composite/GCE [3], graphene-ionic liquid-chitosan composite/GCE [4], silver nanoparticles-polydopamine-graphene nanocomposite modified GCE [21] and poly-L-cysteine/zinc oxide nanoparticles electrospun copper oxide nanofibers based graphite electrode [22] have been reported. However, the major challenges lie in the electrochemical sensors is complex material preparation, low stability, less reproducibility, and high background current. To the best of our knowledge, detection of GU and AD using Fe₂V₄O₁₃ NPs modified carbon paste electrode has not been reported.

In this connection, we have proposed a novel sensing platform for the determination of GU and AD using vanadium based metal oxide nanoparticles modified carbon paste electrode (Fe₂V₄O₁₃NPs/CPE) which is beneficial in terms of simple synthetic protocol, high surface to volume ratio, high conductivity, electrocatalytic activity and surface renewability for the repetitive measurement of the target analytes. Additionally, Fe₂V₄O₁₃ has also attracted growing interest in electrochemical sensor owing to its crystal structure resulting from the vanadium-oxygen polyhedra linkages (Figure S1).

Several methods including electrostatic spray deposition, hydrothermal techniques, precipitation and flux melt method were developed to prepare pure Fe₂V₄O₁₃ [23–26]. The reported methods usually associated with complicated procedures like long synthesis time and heating at higher temperature, which actually hinders the use for practical applications. Therefore, in this paper, Fe₂V₄O₁₃ NPs have been prepared according to our previous method which operates relatively at lower temperature, where sucrose has been used as an oxidizer and ammonium vanadate as vanadium source [27]. The prepared Fe₂V₄O₁₃ NPs were used for the fabrication electrochemical sensor to detect AD and GU wherein the proposed sensor exhibit nanomolar detection limit with good sensitivity and selectivity.

2 Experimental

2.1 Material and Methods

AR grade Guanine and Adenine, nujol oil, graphite powder sucrose, sucrose and ammonium vanadate were purchased from Merck, India, and used without further purification. Phosphate buffer (PB), Britton-Robinson (B–R) and acetate buffer (HAc–NaAc) solution were prepared in distilled water. 0.1 M phosphate buffer solution (PBS) of pH ranging from 5.8 to 8.0 was prepared by mixing potassium dihydrogen phosphate and dipotassium hydrogen phosphate in an appropriate ratio. 10 mM of standard analytes of GU and AD were prepared by using 0.1 M NaOH.

2.2 Synthesis of Fe₂V₄O₁₃ Nanoparticles

Ammonium vanadate (4 mmol) was dissolved in minimum quantity of water at 70°C. Ferric nitrate (8 mmol) was subsequently added to the vanadate solution and stirred for 10 minutes. To the resulting homogeneous mixture, sucrose was added and continued stirring for 10 minutes to ensure homogeneous mixing. Finally, the beaker containing homogeneous mixture was placed in a pre-heated muffle furnace maintained at 350 ± 10°C for 1 hours. The resultant powder was crushed with mortar and pestle, and stored for further studies.

2.3 Instrumentation

X-ray powder diffraction (PAnalytical X’pert PRO X-ray diffractometer) was used to analyze the phase purity, and crystallinity of the prepared Fe₂V₄O₁₃ NPs. The specific surface area, pore size and pore diameter of Fe₂V₄O₁₃ NPs were determined by nitrogen adsorption/desorption at 77 K based on Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) using Quanta chrome correlative NOVA 1000. Fourier transform infrared (FTIR) spectra of Fe₂V₄O₁₃ NPs were monitored by Perkin-Elmer FTIR spectrometer (Spectrum 1000) using KBr pellets over the range of 400–4000 cm⁻¹. The absorption spectrum of Fe₂V₄O₁₃ NPs was measured by SL 159 ELICO UV-visible absorption spectroscopy. The morphology, particles size and crystallinity of Fe₂V₄O₁₃ NPs were analyzed by using FEI Tecnai F-30 transmission electron microscope (TEM).

The electrochemical experiments were performed with SP-150 electrochemical workstation utilizing CV, DPSV and impedance spectroscopic (EIS) techniques. The conventional three electrode electrochemical cell with platinum wire, calomel and carbon paste (modified and unmodified) electrodes served as an auxiliary, reference and working electrodes respectively.

2.4 Fabrication of Fe₂V₄O₁₃ Nanoparticles Modified Carbon Paste Electrode

The unmodified carbon paste electrode (CPE) was prepared by mixing of graphite powder, nujol oil (80:20) in an agitate mortar and ground until to get a homogeneous paste. The resulting homogeneous paste was packed into the cavity of 3 mm dia Teflon tube and electrical contact was made by using copper wire. The electrode surface was polished and smoothened using soft paper and then rinsed carefully with double distilled water prior to each experiment [28]. Similarly, modified carbon paste electrode was prepared by mixing the graphite powder, nujol oil and Fe₂V₄O₁₃ NPs in the ratio60:20:20 (w/w).

2.5 Sample Preparation of DNA

Thermally denaturation of herring sperm DNA was prepared according to the previous literature [29–31]. In
brief, the DNA sample was dissolved in de-ionized water and heated 100°C for 20 minutes and finally cooled rapidly in an ice-water bath. The solution was then adjusted to neutral with 1 M NaOH and diluted with the supporting electrolyte.

2.6 Milk Powder and Urine

The milk powder sample was purchased from the local supermarket and its pretreatment process was carried out according to the previous report [3,32]. In brief, 1.0 g of milk powder was dissolved in 10 mL of water. To this, 5.0 mL of acetic acid (3%, v/v) was added and allowed for 15 min. Then, it was diluted to 20.0 mL with de-ionized water. Finally, the centrifuged for 10 min at 4000 rpm and then the supernatant liquid was collected. The urine sample was obtained from the healthy volunteer and used without further purification. Blood sample was obtained from the local hospital and the sample for electrochemical measurements were prepared according to the previously reported literature [32].

3 Results and Discussion

3.1 Crystallinity, Morphology and Surface Properties of Fe₂V₄O₁₃ Nanoparticles

The powder X-ray diffraction pattern of powder sample prepared at 350°C/1 h is shown in Figure 1a, wherein all the diffraction peaks corresponds to well-crystallized monoclinic phase of Fe₂V₄O₁₃ (JCPDS 87–1845). The average crystallite size (D) was examined using the Scherer formula, D = Kλ/βcosθ. The FWHM of the (002) diffraction peak was fitted using Gauss function (Inset of Figure 1(a)) and the FWHM of the true (002) diffraction peak was estimated using the equation β² = B²−b², where

Fig. 1. (a) X-ray diffraction pattern, (b) TEM images (c) SAED pattern and (d) FTIR spectrum of Fe₂V₄O₁₃ nanoparticles.
The FWHM of the sample and reference respectively [33]. The average crystallite size was found to be ~34 nm.

The TEM image and SAED pattern of the prepared Fe₂V₄O₁₃ NPs are shown in Figure 1b and 1c respectively. Fe₂V₄O₁₃ NPs have uniform diameters with the crystallite size of ~35 nm. The bright diffraction spots observed in the SAED pattern (Figure 1c) reveals the highly crystalline nature of Fe₂V₄O₁₃ NPs [34].

The formation of phase pure Fe₂V₄O₁₃ NPs were further confirmed by FTIR spectrum. The FTIR spectrum of Fe₂V₄O₁₃ NPs prepared at 350 °C for 2 hours is shown in Figure 1d. The bands appeared at 523 cm⁻¹ and between 1050 and 850 cm⁻¹ are attributed to the V–O–V deformation and V–O terminal stretching mode respectively. Additionally, the bands appear between 850 and 550 cm⁻¹ correspond to V–O–Fe bridging and V–O–V stretching mode. The observed result is consistent with the FTIR spectrum of Fe₂V₄O₁₃ reported by Surca et al [35].

The N₂ adsorption-desorption isotherm and BJH pore size distribution of Fe₂V₄O₁₃ NPs are shown in the Figure 2a and 2b respectively. The Fe₂V₄O₁₃ NPs display type IV isotherm with specific surface area, calculated based on BET, of 26 m²g⁻¹. The pore size distribution obtained from desorption isotherm, shown in Figure 2b, exhibit wide distribution between 76 Å and 145 Å, suggesting the presence of mesopores in the prepared samples.

The UV-visible diffuse reflectance spectrum of the Fe₂V₄O₁₃ NPs is shown in Figure 2c. It has been observed that the absorption edge extends from ultraviolet to the visible-light region. The direct band gap calculated based on the equation \( a\nu v = A(h\nu - E_g)^{3/2} \) is found to be 1.84 eV (Figure 2d), which is in good agreement with the reported Fe₂V₄O₁₃ nanoribbons [36]. Thus, small crystallite size with mesoporous structure and semiconducting nature of Fe₂V₄O₁₃ NPs might be an advantageous for bio-molecules sensing applications [34].

Fig. 2. (a) N₂ adsorption-desorption isotherm (b) Pore size distribution, (c) UV-Visible spectrum and (d) Band gap of Fe₂V₄O₁₃ nanoparticles.
3.2 Electrochemical Characterization

Electrocatalytic behavior of Fe$_2$V$_4$O$_{13}$ NPs/CPE has been examined using CV technique in presence of standard redox probe [Fe(CN)$_6$]$_{3-4}$ containing 0.01 M KCl as a supporting electrolyte. Figure 3a represents the typical CVs response of unmodified CPE and Fe$_2$V$_4$O$_{13}$ NPs/CPE where the peak-to-peak separation ($\Delta E_p$) for unmodified CPE and Fe$_2$V$_4$O$_{13}$ NPs/CPE were found to be 128 mV and 113 mV respectively. The obtained result concludes that the presence of electrocatalytically active Fe$_2$V$_4$O$_{13}$ NPs enhances the electron transfer kinetics rate at the electrode interface. Further, the change in magnitude of peak current for redox probe has been improved at Fe$_2$V$_4$O$_{13}$ NPs/CPE over the unmodified CPE. The improved analytical response of the proposed sensor is due to the catalytic effect of Fe$_2$V$_4$O$_{13}$ NPs, which intern enhance the surface area of the composite material on the electrode surface and also provides a feasible pathway for the electron transfer between the electrode interfaces to the bulk of the electrolytic solution [37]. The active surface area of the electrode, calculated based on Randles-Sevcik relation [38], is found to be 0.092 cm$^2$ and 0.127 cm$^2$ for unmodified CPE and Fe$_2$V$_4$O$_{13}$NPs/CPE respectively.

The EIS analysis was carried out to confirm the electrocatalytic nature of the electrode surface area. The electrochemical impedance spectrum is an important platform to understand the charge transfer resistance of the surface-modified electrodes. Figure 3b represents the Nyquist plots of the EIS of unmodified CPE (black line) and Fe$_2$V$_4$O$_{13}$ NPs/CPE electrode (red line) using the ferrocyanide as a model. The presence of a semicircle at high frequency region in the Figure 3b indicates the electron transfer process and the linear part at low frequency region indicates the diffusion process. The diameter of the semicircle represents the electron transfer resistance ($R_{ct}$) at the electrode [39]. The charge transfer resistance value for bare unmodified CPE and Fe$_2$V$_4$O$_{13}$ NPs/CPE was found to be 592.6 and 456.7 $\Omega$, respectively. As compared with bare CPE, the diameter greatly decreased with the modification of Fe$_2$V$_4$O$_{13}$ NPs on CPE, which is ascribed to the reduced electron transfer resistance. The observed results illustrate that Fe$_2$V$_4$O$_{13}$NPs/CPE has good conductivity on electro-oxidation of AD and GU.

3.3 Electrocatalytic Activity of Fe$_2$V$_4$O$_{13}$ NPs Modified CPE towards Oxidation of GU and AD

The preliminary investigation of the unmodified CPE and Fe$_2$V$_4$O$_{13}$ NPs/CPE towards analytical response in the presence and absence of AD and GU individually and simultaneously at physiological condition using CV and the results of which are presented in Figure S2. It is observed that GU and AD alone and mixture of these two exhibit only oxidation peaks and thereby suggesting the irreversible nature.

Further, electrocatalytic response of unmodified CPE and Fe$_2$V$_4$O$_{13}$ NPs/CPE has been investigated in detail using DPSV as shown in the Figure 4a–c. From the Figure 4, the unmodified CPE showed lesser peak current compared to the Fe$_2$V$_4$O$_{13}$ NPs/CPE at a peak potential of 0.64 V and 0.93 V for GU and AD, respectively. The obtained results are in good agreement with the reported literature [40]. Besides, Figure 4c showed a well-defined peak to peak separation with improved analytical response for both GU and AD. The increase in the peak current and minimization in the peak potential is ascribed due to the presence of Fe$_2$V$_4$O$_{13}$ NPs upon the electrode surface, which promotes the electrode kinetics [41].

The enhancement in the peak current for the oxidation of GU and AD is due to the presence of Fe$_2$V$_4$O$_{13}$ NPs within the carbon paste matrix which improves the rate of
electron transfer between the electrode interface and bulk of the electrolyte solution which in turn greatly enhanced the conductivity of the proposed interface. Nevertheless, the improved electrochemical activity of the proposed sensor could be attributed to the formation of double layer across the interface, which is the result of negative and positive surface charges of Fe₂V₄O₁₃ NPs and electrolytes where the negative surface charge increases the adsorption of GU and AD. Contrary, in case of unmodified CPE, the observed result was not much significant compared to Fe₂V₄O₁₃ NPs/CPE. Hence, the proposed sensing platform could be used as promising candidate for the simultaneous detection of GU and AD.

3.4 Effect of Scan Rate and Determination of α and k₀

The electrochemical oxidation of GU and AD at Fe₂V₄O₁₃ NPs/CPE has been recorded using CV under identical conditions which provide a mechanistic approach of the modified interface towards target analytes in terms of electrode kinetics. The observed anodic peak currents of GU and AD at Fe₂V₄O₁₃ NPs/CPE showed an increase in peak current with increase in the scan rate over the range 10 to 100 mV s⁻¹ (Figure 5).

A linear relationship between the oxidation peak current and scan rate was expressed in regression equations as GU: \( I_{\text{pa}} = -48.76 \nu (\text{Vs}^{-1}) + 1.434\); \( R^2 = 0.9750 \) and AD: \( I_{\text{pc}} = -15.88 \nu (\text{Vs}^{-1}) + (-0.83); \ R^2 = 0.9528 \) (Inset of Figure 5). From the plot of \( \log I_{\text{pa}} \) vs. \( \log \nu \), for GU and AD, the slope values were close to 1.0, which holds good for the oxidation process of surface bound species in terms of adsorption controlled [18]. Further, the irreversibility of the modified interface has been confirmed by shift in the peak potential towards more positive window with respect to scan rate. A linear relationship between peak potential (\( E_{\text{p}} \)) and logarithmic scan rate (\( \ln \nu \)) could be
expressed by the following regression equation; GU: $E_p = 0.0202 \ln(n) + 0.6716$; $R^2 = 0.9333$ and AD: $E_p = 0.0278 \ln(n) + 0.9343$; $R^2 = 0.9352$.

The $E_p$ for an irreversible electrode process was given by Laviron’s relation [42],

$$E_p = E^0 + \left(\frac{RT}{nF}\right) \ln \left(\frac{nF}{RTk_0n}\right) + \left(\frac{RT}{nF}\right) \ln \nu$$

Where, $E^0$ is formal potential and $k_0$ is the standard heterogeneous reaction rate constant; $n$ is the number of electron transferred; $\nu$ refers to charge transfer coefficient; $R$, $T$ and $F$ have their usual meaning. The value of $n$ could be obtained from the slope of $E_p$ vs. $\ln\nu$, and was found to be 0.0202 and 0.0278 for GU and AD respectively, taking $T=298$ K, $R=8.314$ JK$^{-1}$ mol$^{-1}$, and $F=96480$ Cmol$^{-1}$, $n$ were calculated to be 1.021 and 0.923 for GU and AD, respectively. Generally, $\alpha$ was assumed to be 0.5 in a total irreversible electrode process. Therefore, the number of electrons (n) transferred during electro-oxidation of GU and AD was calculated to be $\approx 2$. The electro-oxidation of GU and AD at Fe$_2$V$_3$O$_{12}$NPs/CPE were two-proton and two-electron process which is in good agreement with the reported literature [43].

### 3.5 Optimization of Experimental Parameters

The sensitivity of the proposed Fe$_2$V$_3$O$_{12}$ NPs/CPE could be enhanced by optimizing the various analytical parameters in an aqueous medium utilizing pH of the buffer solution, pre-concentration time and pre-concentration potential which influences the analytical activity in terms of the electron transfer between the electrode interface and the electrolytic solution.

#### 3.5.1 Effect of Supporting Electrolytes and pH

The sensitivity of the proposed Fe$_2$V$_3$O$_{12}$NPs/CPE has been verified in various supporting electrolytes such as PBS, HAc–NaAc and B–R buffer solution (Figure S3). Among these, PBS show good analytical response at physiological condition. Hence, PBS was used as a supporting electrolyte for further experiments.

The effect of protonium ion concentration on the peak current response of GU and AD at Fe$_2$V$_3$O$_{12}$NPs/CPE were recorded using DPSV in PBS. Figure 6a represents the DPSVs of GU and AD at Fe$_2$V$_3$O$_{12}$NPs/CPE with PBS, the pH varied from 5.4 to 8.0. As shown in the figure, better current response and well defined peak shape in DPSV were observed at pH 7.4. Hence, the optimum pH of 7.4 was chosen for further investigations. Figure 6b shows the relationship between the peak potentials and pH where it is found to be linear with the linear regression equations of GU and AD were expressed as follows $E_p (V) = -0.053 \text{pH} + 1.059$ (R$^2 = 0.9352$) for GU and $E_p (V) = -0.025 \text{pH} + 1.059$ (R$^2 = 0.9333$) for AD.
$0.9156$ and $E_p (V) = -0.055 \text{pH} + 1.371 \ (R^2 = 0.9213)$, respectively. These slope values were closed to the theoretical value of $-0.059 \text{V/pH}$ at $25^\circ C$, as expected from the Nernst equation, which suggested that each molecule of GU and AD were involved in the equal number of protons and electrons [44] during electrochemical oxidation process.

### 3.5.2 Effect of Accumulation Potential and Time

The effect of accumulation time and accumulation potential on the stripping peak current of GU and AD has been studied by using DPSV. The effect of accumulation potential on anodic peak current response of GU and AD was carried between $-1.0$ to $0.4 \text{ V}$ and the result of which was shown in Figure 7a. From the Figure, the anodic peak current for GU and AD were almost unchanged with respect to the various accumulation potential [45]. As part of the better analytical sensitivity, $-0.5 \text{ V}$ was chosen as an optimal accumulation potential for further experimental studies.

As the accumulation time promotes the target analytes to get adsorbed on the electrode interface which would greatly help to achieve the better electrochemical response. Hence, the accumulation time has been studied over the range from 0 to 180 s. Figure 7b depicts that, the stripping peak current gradually increased from 0 to 150 s, which is due to the more and more analyte will re-oxidize and thereby strip back in to the bulk of the electrolytic solution. Hence, better analytical current could be expected. Further, the slight decrease in the peak current after 150 s is due to the surface saturation of the Fe$_2$V$_4$O$_{13}$NPs/CPE [46]. Therefore, the optimal accumulation time of 150 s was preferred for further studies.

### 3.5.3 Effect of Fe$_2$V$_4$O$_{13}$ NPs Content on the Electrode

The effect of Fe$_2$V$_4$O$_{13}$ NPs content (5 to 30% in paste) for the analytical response of GU and AD (20 $\mu$M each) in PBS (pH 7.4) were studied using DPSV and the observed results were shown in Figure S4. From the Figure it is evidenced that the Fe$_2$V$_4$O$_{13}$ NPs content in the paste up to 20% exhibited the highest oxidation current for GU and AD. The improved analytical response of the Fe$_2$V$_4$O$_{13}$ NPs is due to the large surface area and mesoporous together with semiconducting nature. Further increase in the Fe$_2$V$_4$O$_{13}$ NPs content leads to decrease in the current response which might be due to the surface saturation of the electrode. Therefore, 20% Fe$_2$V$_4$O$_{13}$ NPs content was used for further studies.

### 3.6 Individual and Simultaneous Detection of GU and AD

The potential affinity of the Fe$_2$V$_4$O$_{13}$ NPs/CPE towards GU and AD was investigated under optimized electrochemical conditions utilizing DPSV. Figure 8a and 8b represents the anodic peak currents of GU and AD recorded by keeping the concentration constant either one of the analyte. The electrochemical response of GU and AD were gradually increased which is proportional to the concentration of the target analytes by keeping the concentration of the other analyte constant. The current response with respect to the concentration of the target analyte has been given in the form of linear regression equation and it was found to be $I_{pa} (\mu A) = 2.095 + 1.268 \ [\text{GU}] \ (\mu M); R^2 = 0.9644 \text{ for GU (Figure 8d) and } I_{pa} (\mu A) = 2.678 + 1.434 \ [\text{AD}] \ (\mu M); R^2 = 0.9871 \text{ for AD (Figure 8e)}$. The slopes correspond to the sensitivity of the Fe$_2$V$_4$O$_{13}$NPs/CPE and it was found that 1.268 $\mu$A/$\mu$M for G and 1.434 $\mu$A/$\mu$M for A. Based on the linear regression equation, the limit of detection (LOD = $3S_b/m$, where $S_b$ is standard deviation of nine blank determinations and $m$ is
The selectivity of the Fe$_2$V$_4$O$_{13}$ NPs/CPE towards the GU and AD has been investigated simultaneously. Figure 8c shows that the overlaid DSPV of GU and AD over the concentration range 0.5 to 100 μM. From the Figure 8c, with increasing the concentration of the analytes the stripping peak current also increased over a wide linear range. Further, the calibration graph has been constructed and is shown in Figure 8f. The linear regression equation of AD and GU was respectively found to be, $I_{pa}$ (μA) = $-2.185 + 1.393 [GU]$ (μM); $R^2 = 0.9626$ and "slope of calibration plot) for GU and AD were found to be 0.039 and 0.042 μM respectively.

Fig. 8. DPVs of (a) AD (constant) with varying GU concentration, (b) GU (constant) with varying AD concentration, (c) varying both AD and GU concentration using Fe$_2$V$_4$O$_{13}$ NPs/CPE, (d,e,f) Calibration plot of GU, AD and both GU and AD respectively.
Table 1. Performance comparison of the G and A sensors created in this study with those reported in the literature.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Methods</th>
<th>Linear range (µM)</th>
<th>Detection limit (µM)</th>
<th>References</th>
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<td>NMP-Exfoliated-GCE</td>
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<td>DPSV</td>
<td>0.5–100</td>
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</tr>
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</table>

NMP (N-methyl-2-pyrrolidone); PANI/MnO₂ (Polyaniline-manganese dioxide); Carbon nanotube-poly(new fuchsins); CCE: carbon ceramic electrode; MWCNT/GCE: GCE modified with carboxylated MWCNTs, CILE-carbon ionic liquid on the carbon paste electrode, PTFSA-Poly(para toluene sulfonic acid), rG (Reduced graphene).

\[ I_{pa} (\mu A) = -2.962 + 1.851 \ [AD] (\mu M); R^2 = 0.9493. \]

The slope of regression equations from the calibration graph of each species and mixture of species was found to be nearly equal; these results conclude that they did not interfere during their mutual determination.

The LOD (3σ) was found to be for GU and AD was 0.032 and 0.037 µM with corresponding sensitivity of 1.393 and 1.851 µA/µM respectively. The observed limit of detection is comparatively better than the recently reported electrochemical sensor based on PbTe nanocrystals [40]. The reason for getting such a low detection of the proposed sensor is mainly due to the presence of more number of trapping sites upon the nanoparticles surface along with large surface to volume ratio and mesoporous structure which in turn enhances the analytes to be get adsorbed on the electrode interface and thereby promote better electron transfer between the electrode interface and the bulk of the solution [47]. Hence, the proposed electrochemical sensor could replace the existing ones in order to determine the GU and AD either individually or simultaneously in real sample matrices. The proposed electrochemical sensing tool has been compared with the other existing sensors as shown in Table 1, where one can notice that the proposed sensor exhibits wide linear range and good limit of detection compared to the existing sensors.

### 3.7 Reproducibility and Stability of the Fe₂V₄O₁₃ NPs/CPE

Ten Fe₂V₄O₁₃ NPs/CPE prepared from the same way were used to investigate the reproducibility by detecting the 20 µM of GU and AD. The relative standard deviation (RSD) for the oxidation currents of GU and AD were 2.76% and 3.12%, respectively, showing good reproducibility of the biosensor.

The stability of the biosensor was demonstrated by keeping the Fe₂V₄O₁₃ NPs/CPE for 30 days at room temperature. During that time, the Fe₂V₄O₁₃ NPs/CPE was applied to detect 20 µM of GU and AD. The analytical performances showed the RSD 3.3% and 3.5%, for GU and AD, respectively, which proved the electrode possessed high stability.

### 3.8 Analytical Applications

In order to evaluate the practical applicability of the proposed sensor, the Fe₂V₄O₁₃ NPs/CPE was successfully applied towards the simultaneous measurement of GU and AD content in thermally denatured DNA. The simultaneous detection of GU and AD concentration was performed by standard addition method [18]. In brief, 50 µL of the thermally denatured DNA was added to the electrochemical cell containing phosphate buffer (pH 7.4) solution and the current response of GU and AD was recorded using DPSV. The observed result illustrates that the presence of two-well resolved anodic peaks refers to the oxidation of GU and AD in denatured DNA. Further, a known aliquot of GU and AD were added to the same electrochemical cell to measure the oxidative peak currents of GU and AD. The concentration of GU and AD were calculated from the difference between the oxidation peak currents in thermally denatured DNA and standard solution and it was found to be 0.17 and 0.28 respectively. Additionally, the proposed sensor has been successfully applied to real samples such as milk powder and urine to determine the content of GU and AD. Under identical/optimized conditions, five times measurements were performed. The obtained results were tabulated in Table 2 and achieved good recoveries for the GU and AD samples.

### 4 Conclusions

In summary, a simple electrochemical sensor based on Fe₂V₄O₁₃ NPs/CPE was fabricated for the sensitive and simultaneous nanomolar determination of GU and AD. The Fe₂V₄O₁₃ NPs was prepared using a simple and facile solution combustion method. The proposed sensor exhibi-
its a wide linearity between the concentration 0.5 and 60 μM, and limit of detection (3σ) for GU and AD is found to be 32 and 37 nM. The wide linearity and lower detection limit could be attributed to presence of abundant electroactive sites on nano-sized Fe₂V₄O₁₃, which greatly improved electron transfer amongst the analytes and the electrode interface. The proposed Fe₂V₄O₁₃ NPs/CPE sensor shows good stability and acceptable reproducibility. The proposed simple Fe₂V₄O₁₃ NPs/CPE could be used as a feasible biosensor for the determination of biomolecules in clinical diagnosis.

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