

An Electrochemical Sensor for the determination of Uric Acid based on CdSe Quantum Qots (QDs) / Graphene Oxide (GO) nanocomposite

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Abstract – A chemically modified electrode has been constructed based on CdSe QDs/GO nanocomposite and employed for the selective determination of Uric acid (UA) in 0.1M Phosphate buffer solution, (PBS, pH 7.0). Field emission scanning electron microscope (FESEM) was applied to characterize the surface morphology and the performance of the modified electrode was examined by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The results showed that the electrochemical sensor showed excellent electrocatalytic activity towards the oxidation of UA. Under optimum conditions, the linear range for the detection of UA is 1.7×10^{-6} M to 1.0×10^{-4} M and the detection limit was found to be 5.6×10^{-7} M (S/N = 3). The utility of the modified electrode in flow systems for the determination of UA was evaluated by hydrodynamic and chronoamperometric studies. The high sensitivity, selectivity, reproducibility and long-term stability of the CdSe QDs/GO nanocomposite modified electrode provided a sensing platform for the determination of UA.

Keywords: CdSe QDs, Graphene Oxide, Nanocomposite, Uric Acid

I. INTRODUCTION

Uric Acid (UA) is an important final product of purine metabolism in the human body and it is produced from the cellular breakdown products of the purine nucleosides, adenosine and guanosine [1]. The normal physiological level of UA in human body ranges from 0.3 to 0.5 mM in serum and 1.4 to 4.4 mM in urinary excretion [2]. Abnormal levels of UA are the symptoms of several diseases such as gout, hyperuricaemia and Lesch-Nyhan syndrome [3, 4]. Therefore, it is essential to develop simple and rapid method for the determination UA in biological fluids. Various methods have been developed for the determination of UA in biological samples, including colorimetry [5], spectrophotometry [6], chromatography [7], chemiluminescence [8], fluorescence [9], and electrochemistry [10]. However, some of these methods are very expensive or complicated and time-consuming and are unsuitable for field-monitoring. Generally, electroanalytical techniques having advantages of easy fabrication, more selective, fast response, less time-consuming and portable nature are more suitable for the detection of biomolecules.

Nanomaterials in fabricating sensors have attracted considerable attention because of their unique properties of large surface area, chemical stability and conductivity. Among the nanomaterials, Quantum dots (QDs), which are semiconductor nanoparticles containing group II and VI elements have received overwhelming interest by virtue of their excellent properties like, magnetic, electronic, catalytic and optical which are suitable in various fields [11, 12]. QDs offers many advantages like high stability, selectivity and sensitivity, excellent catalytic activity in the construction of electrochemical sensors [13, 14]. Also, Graphene oxide (GO) one of the important derivative of graphene has large surface area, excellent conductivity, high stability and good mechanical strength. The presence of defects in GO changes the electronic and chemical properties [15]. GO based electrochemical sensors offer advantages by lowering the detection limit, ability to decrease the over potential, improve the selectivity and high stability [16, 17].

In this work, we present the fabrication and application of modified electrode by immobilizing CdSe QDs/GO nanocomposite for the sensitive determination of UA. The nanocomposite was prepared by ultrasonication method. The structural features of the prepared CdSe QDs and CdSe QDs/GO nanocomposite was examined by FESEM. CdSe QDs/GO nanocomposite modified electrode was prepared by simple dropcasting method. The nanocomposite modified electrode was electrochemically characterized by CV and EIS. The analytical performance of the sensor was evaluated by CV and chronoamperometry. The modified electrode could remarkably improve the electrochemical response of UA. The modified electrode separated the voltammetric signals of UA and L-dopa efficiently, showing excellent selectivity of the modified electrode.

II. EXPERIMENTAL

a. Chemicals and reagents

Spectroscopic grade graphite rod (3 mm), graphite powder (1-2 μM) and cadmium chloride hemi penta hydrate were purchased from Aldrich, Germany. L-cysteine ethyl ester hydrochloride, uric acid and L-dopa were purchased from Hi-media. All other chemicals and reagents were of analytical grade and used without further purification. All aqueous solutions were prepared with doubly distilled water. Phosphate buffer solution (PBS 0.1 M) of pH 7.0 was employed as supporting electrolyte for all the electrochemical measurements.

b. Instruments

FESEM images were obtained using Hitachi SU-6600 microscope (Japan). All the voltammetric determinations were carried out with a CHI 660B electrochemical workstation (CH Instruments, USA). A conventional three-electrode system was used that consisted of the CdSe QDs/GO nanocomposite modified as the working electrode, a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE). pH measurements were made with an Elico pH meter (model LI 120, India). All experiments were carried out at ambient temperature.

c. Preparation of CdSe QDs

L-Cysteine capped CdSe QDs were synthesized according to the reported procedure [18] with a slight modification. 20 mL aqueous solution of 3.2×10^{-4} M CdCl_2 and 3.2×10^{-3} M L-cysteine ethyl ester hydrochloride were added into a three-necked round bottom flask and the pH was adjusted to 11 using 1 M NaOH. Then it was added with 20 mL of NaHSe, which was prepared by adding 3.2×10^{-3} M of Selenium metal and 8.1×10^{-2} M of sodium borohydride in an inert atmosphere. The mixture was stirred for 30 minutes and then refluxed for 4 hours under nitrogen atmosphere. After 4 hours an orange red colloidal CdSe QDs was formed

d. Preparation of Graphene Oxide

Graphene oxide was prepared by modified Hummers method [19]. In short, graphite powder (1.0 g), NaNO_3 (1.0 g) and 46 mL of concentrated H_2SO_4 (98%) were added into a beaker under stirring kept in an ice bath. Then, 6.0 g of KMnO_4 was slowly added to the mixture and was vigorously stirred below 20°C . After 1 h stirring at room temperature, the solution was diluted with 70 mL of water and then stirred at 95°C for 2 h. The mixture was then added with 100 mL of water and 30 mL of 30% H_2O_2 and stirred for 30 minutes. Then, it was centrifuged and dried under vacuum to get the GO. About 0.5 mg of GO was ultrasonically dispersed in 1 mL of ethanol to get 0.5 mg/mL dispersion of GO.

e. Preparation of CdSe QDs / GO nanocomposite modified electrode

The modified electrode was fabricated as reported in our previous work [20]. The CdSe QDs/GO nanocomposite was prepared by ultrasonically dispersing 60 μL of CdSe QDs with 1 ml ethanol containing 0.5 mg of GO for 30 min. PIGE was prepared as reported [21] and used for electrode modification. One end of the electrode was carefully polished with emery paper and then with $0.05\mu\text{m}$ alumina slurry, washed with distilled water and dried in air. Then, 5 μL of the suspension was drop casted on to the mirror polished surface of PIGE and dried at room temperature to get CdSe QDs/GO nanocomposite modified electrode. For comparison, 5 μL of CdSe QDs colloidal solution was dropcasted on a PIGE to get a CdSe QDs modified electrode and 5 μL of GO was dropcasted on a PIGE to get a GO modified electrode.

III. RESULTS AND DISCUSSION

a. Characterization of CdSe QDs and CdSe QDs/GO nanocomposite

The surface morphology of CdSe QDs and CdSe QDs/GO nanocomposite were examined by FESEM. Fig.1 (A) shows the FESEM micrograph of CdSe QDs showing the spherical morphology and is present in the L-cysteine network. The size of the particles ranges from 5-20 nm. The image of nanocomposite (Fig. 1(B)) exhibits the successive loading of CdSe QDs on GO film. The large surface area of the film helps to load more amount of CdSe QDs.

b. Electrochemical behavior of the CdSe QDs/GO nanocomposite modified electrode

Fig. 2(A) compares the CV response of bare (a), CdSe QDs (b), GO (c) and CdSe QDs/GO nanocomposite (d) modified electrodes in 0.1 M PBS (pH 7.0) at a scan rate of 50 mV/s. As can be seen in the figure, no redox peaks were observed at all the four electrodes indicating that CdSe QDs, GO and CdSe QDs/GO nanocomposite are electroinactive in the selective potential range. However, the background current for CdSe QDs/GO nanocomposite modified electrode was considerably higher when compared to the other electrodes. The results revealed that the combination of CdSe QDs and GO provide great improvement to the increase in the electrochemical response [20].

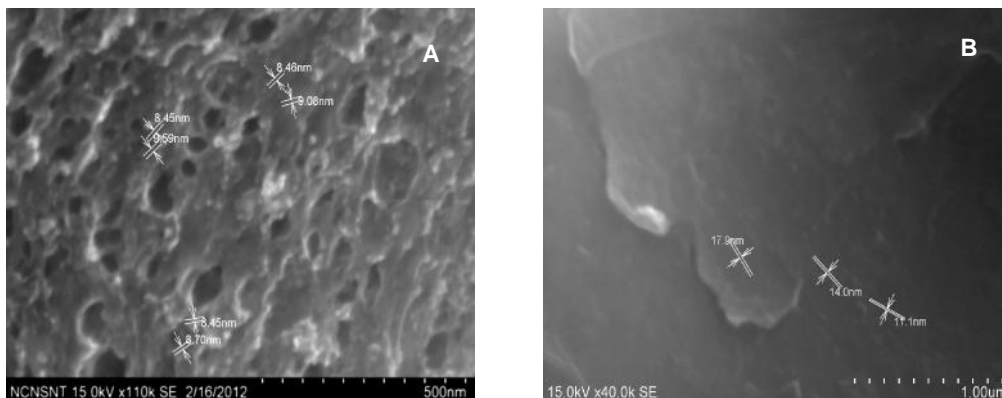


Fig.1 (A) FESEM image of CdSe QDs, (B) FESEM image of CdSe QDs/GO nanocomposite

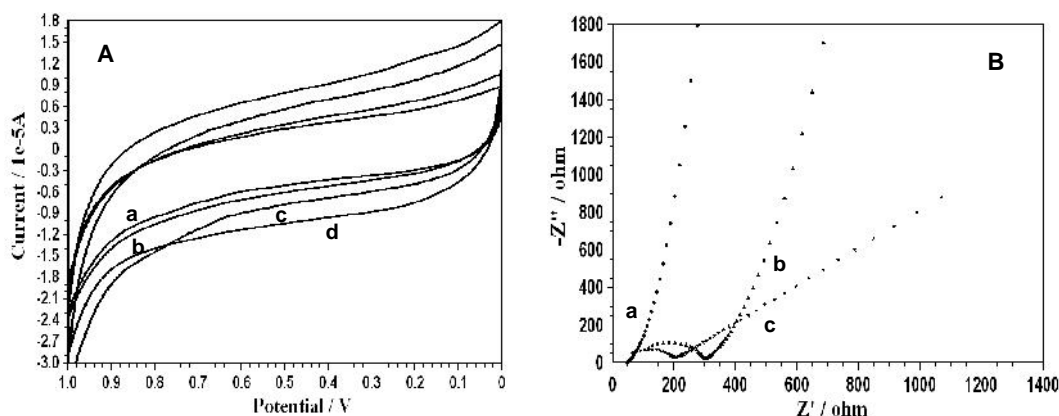


Fig. 2 (A) Cyclic voltammograms obtained at (a) bare, (b) CdSe QDs, (c) GO and (d) CdSe QDs/GO nanocomposite modified electrodes in 0.1 M PBS (pH, 7.0) , scan rate – 50 mV/s (B) EIS of (a) bare, (b) GO and (c) CdSe QDs/GO nanocomposite modified electrodes in 5mM $K_4[Fe(CN)_6]$ with the frequency swept from 1Hz to 0.1 MHz.

EIS was employed to investigate the electron transfer property on the surface of the modified electrodes. Fig. 2(B) shows the typical Nyquist plots for bare (a), GO (b) and CdSe QDs/GO nanocomposite (c) modified electrodes recorded in 0.1 M KCl containing 5mM $Fe(CN)_6^{3-/4-}$ as an electrochemical redox probe. The impedance spectra include a semicircle portion and a linear portion, the semicircle portion observed at higher frequencies corresponds to the electron transfer limited process and its diameter is equal to the charge transfer resistance (R_{ct}). The linear portion appeared at lower frequencies attributed to the diffusion limited electron transfer process. As shown in the figure, the bare electrode exhibited almost straight line which is characteristic of diffusion limiting electron transfer process. When the electrode was modified with GO, the plot showed greater semicircle corresponding to the larger electron transfer resistance, due to the electrostatic repulsion between the negatively charged surface and the probe molecule ($Fe(CN)_6^{3-/4-}$) [22]. Compared to the bare and GO electrode, the CdSe QDs/GO nanocomposite modified electrode showed still lower charge transfer resistance. This is attributed to the synergistic effect of CdSe QDs and it provides good electron conduction pathways between the electrode and electrolyte

The effect of scan rate on the CdSe QDs/GO nanocomposite modified electrode was examined using CV. CVs of the CdSe QDs/GO nanocomposite modified electrode at different scan rates from 5 mV/s to 150 mV/s and the corresponding calibration plot are shown in Fig. 3(A) and (B) respectively. It was observed that both the anodic and cathodic peak currents increased linearly with increase in the scan rate and from the calibration plot the electrode process was found to be diffusion controlled. The electrochemical rugosity (X) or surface concentration of the modified electrode was calculated using the Laviron's Equation $I_p = n^2 F^2 X A \sqrt{4RT}$ [23], where n is the number of

electrons transferred (as $n=1$ for the process $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$), F is the Faraday's constant, A is the area of the electrode, and ν is the scan rate. The X of the modified electrode was calculated to be $1.299 \times 10^{-8} \text{ molcm}^{-2}$.

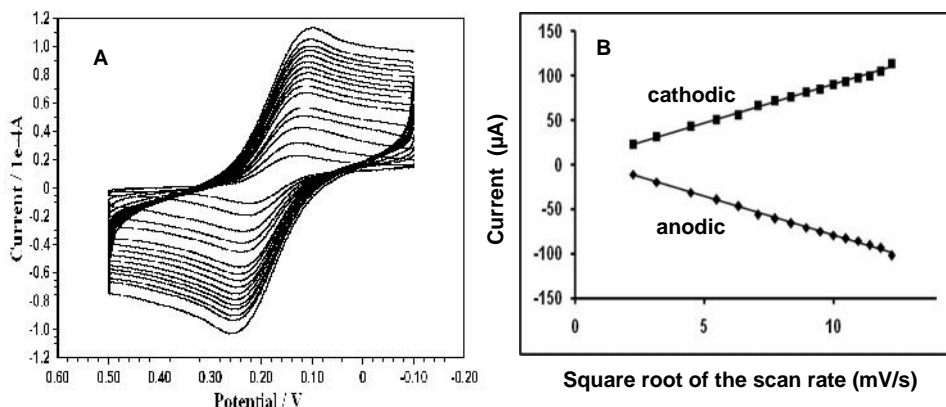


Fig. 3 (A) Cyclic Voltammograms of CdSe QDs/GO nanocomposite modified electrode at different scan rates in 5mM $\text{K}_4[\text{Fe}(\text{CN})_6]$. The scan rates from inside to outer 5, 10 – 150 mV/s with increments of 10 mV/s. (B) Calibration plot of current vs square of scan rate.

c. Electrochemical oxidation of UA at CdSe QDs/GO nanocomposite modified electrode

The CdSe QDs/GO nanocomposite modified electrode exhibits excellent electrocatalytic oxidation of UA. Fig. 4 shows the typical CVs of bare (a), CdSe QDs (b), GO (c) and CdSe QDs/GO nanocomposite (d) modified electrodes in 50 μM of UA in 0.1M PBS (pH 7.0) at a scan rate of 50 mV/s. It is noted that the bare electrode does not show any peak for the oxidation of UA whereas GO modified electrode shows a broad peak at 0.37 V. On the other hand, the CdSe QDs modified electrode exhibits an oxidation peak at 0.31 V. Comparing other electrodes, CdSe QDs/GO nanocomposite modified electrode shows a considerable negative shift in the peak potential at 0.27 V with increase in current response. These results suggest that more efficient oxidation of UA occurs at CdSe QDs/GO nanocomposite modified electrode is probably due to the large surface area and fast electron transfer rate of the nanocomposite.

Fig. 5 (A), (B) shows the CVs of the CdSe QDs/GO nanocomposite modified electrode in the presence of different concentrations of UA and a calibration plot of current response against the concentration of UA. On increasing the concentration of UA, the oxidation peak current was found to be increase. This is attributed to the electrocatalytic activity of the nanocomposite. From the calibration plot, it can be seen that the modified electrode showed a linear response for the oxidation of UA in the concentration range of $1.7 \times 10^{-6} \text{ M}$ to $1.0 \times 10^{-4} \text{ M}$ with a detection limit of $5.6 \times 10^{-7} \text{ M}$ ($S/N = 3$).

d. Amperometric determination of UA at the CdSe QDs/GO nanocomposite modified electrode

Hydrodynamic studies were performed in order to check the applicability of the modified electrode for the determination of UA under dynamic conditions. The current response for the oxidation of UA at a concentration of $1.6 \times 10^{-5} \text{ M}$ was measured as a function of applied potential in the range of 0 – 0.6 V in a stirring solution of 0.1 M PBS at a scan rate of 10 mV/s. Fig. 6 shows a plot of potential vs current of the modified electrode in the presence and in the absence of UA. From the figure, it was perceived that the catalytic oxidation of UA starts at a potential of 0.2 V and attains maximum response at 0.27 V. Hence, an applied potential of 0.32 V was experimentally determined as an optimum potential for amperometric experiments.

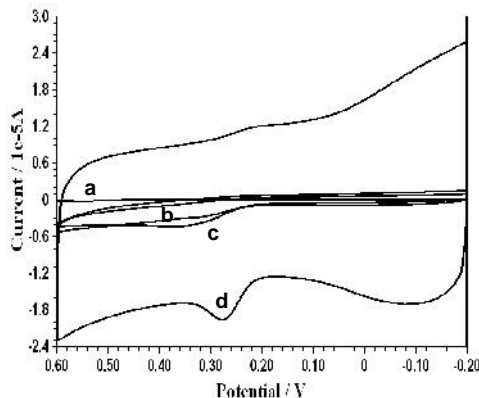


Fig. 4 Cyclic voltammograms 50 μM of UA in 0.1 M PBS at four different modified electrodes (a) bare, (b) CdSe QDs, (c) GO and (d) CdSe QDs/GO nanocomposite, Scan rate – 50 mV/s.

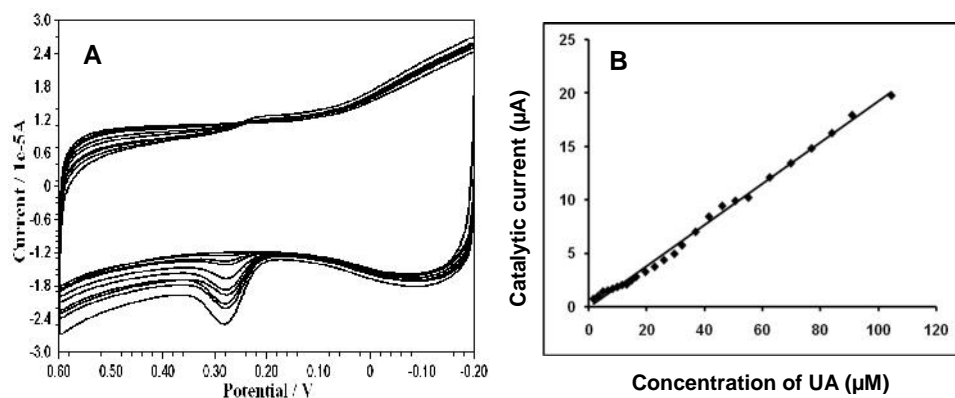


Fig. 5 (A) Cyclic Voltammograms of various concentrations of UA from 0 to 63 μM in 0.1M PBS, Scan rate – 50 mV/s, (B) Calibration plot of catalytic current vs concentration

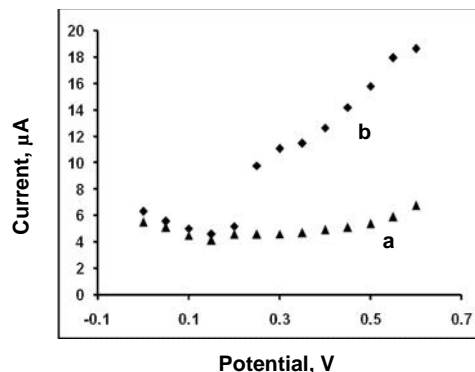


Fig. 6 Hydrodynamic voltammograms of modified electrode (a) modified electrode in the presence of 1.6×10^{-5} M of UA (stirring rate: 300 rpm)

Chronoamperometric response of the CdSe QDs/GO nanocomposite modified electrode for the oxidation of UA was studied at an applied potential of 0.32 V. Fig. 7 shows the current response of the modified electrode in a stirred solution for successive increments of 4 μM of UA. The oxidation current was found to increase with each addition of UA and reaches a steady state within 5 s. This indicates the fact that the modified electrode has a very quick response time for UA determination. Also, a calibration plot of current response vs concentration of UA (Fig. 7A) shows a linear response for the successive increments which indicates that the modified electrode exhibited an excellent catalytic activity towards the oxidation of UA under dynamic condition.

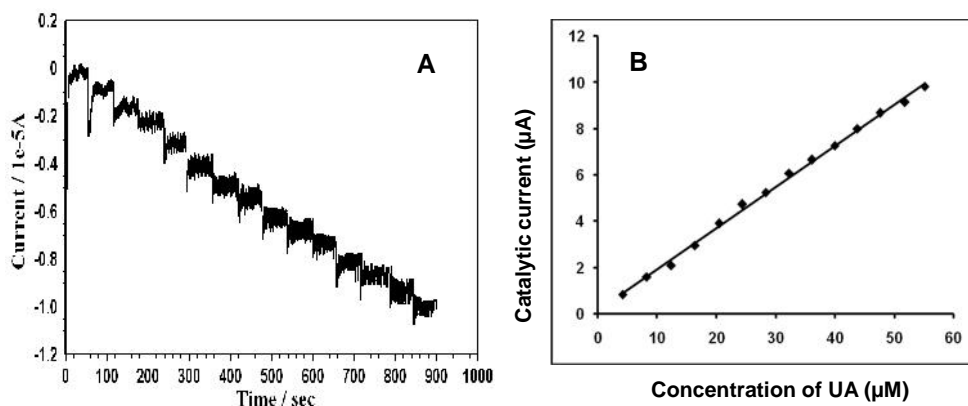


Fig. 7 (A) Amperometric response of CdSe QDs/GO nanocomposite modified electrode for the successive addition of 4 μM of UA in 0.1 M PBS (B) Calibration plot of current response vs concentration

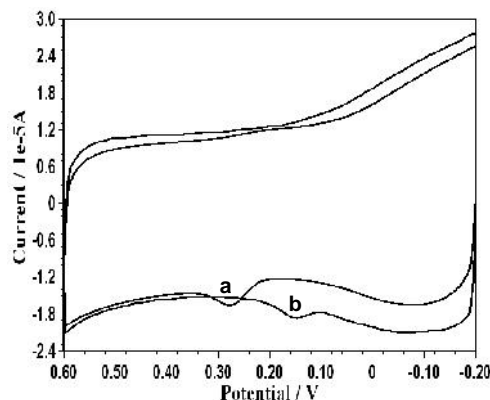


Fig. 8 Cyclic voltammograms of CdSe QDs/GO nanocomposite modified electrode in the presence of 25 μ M of (a) UA and (b) L-Dopa

e. Interference study

The major interference in the determination of UA is L-Dopa and Ascorbic Acid as they coexist together in living systems. Fig. 8 (a) and (b) show the CV response of the CdSe QDs/GO nanocomposite modified electrode in 25 μ M of UA and L-Dopa respectively. As shown in the figure, the potential for the determination of UA is 0.27 V and the potential for the determination of L-Dopa is 0.15 V. This exhibits that L-Dopa does not show any significant effect on the electrocatalytic determination of UA. Also, the modified electrode did not show any response even in the presence of 1mM of AA. This is due to the electrostatic repulsion between the modified electrode surface and ascorbate anion at pH 7. The above results show that the CdSe QDs/GO nanocomposite modified electrode is much reliable and sensitive for the determination of UA in the presence of other interferences.

f. Stability and reproducibility

The practical utility of sensor depends on the stability and reproducibility of the electrode system. In order to evaluate the stability of the modified electrode, it was subjected to 10 consecutive measurements of 50 μ M UA. The oxidation current response of the modified electrode remains within a confidence level of 97%, indicating that the electrode does not undergo any surface fouling during the measurements. The long term stability of the sensor was also studied over a period of 45 days. The electrode was stored in an air tight container when not in use. The modified electrode was able to sense same concentration of UA which showed 96% of the initial current, indicating that the sensor had good long term stability. The fabrication reproducibility of the sensor was evaluated by determining 50 μ M of UA with 5 modified electrodes. The sensor shows excellent reproducibility with a RSD value of 2.8 %. Thus, the sensor showed stable and reproducible response for the determination UA.

IV. CONCLUSION

An amperometric sensor for the determination of UA was fabricated by modifying the electrode surface using CdSe QDs/GO nanocomposite. The prepared nanocomposite was characterized and confirmed by FESEM and HRTEM analysis. The nanocomposite plays a vital role in improving the electron transfer efficiency and thereby enhancing the electrocatalytic activity towards UA determination. The modified electrode can be successfully applied for the selective determination of UA in the presence of excess amount of AA. The proposed modified electrode seems to provide a favorable alternative for the determination UA. The good selectivity and high sensitivity can be achieved by the CdSe QDs/GO nanocomposite makes it a promising candidate for electroanalytical applications.

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