

RESEARCH PAPER

## Fabrication of Nano Poly Cresol Red over Glassy Carbon Electrode and its Application in Selective Determination of Uric acid in the Presence of Ascorbic Acid

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### ABSTRACT

A selective electrochemical method for the determination of uric acid was developed by using nano poly cresol red modified glassy carbon electrode. This new material has been characterized by Scanning Electron Microscopy, cyclic voltammetry and Differential pulse voltammetry. This modified electrode shows excellent electrocatalytic activity towards the oxidation of uric acid in the presence of ascorbic acid in (pH 7.0). Compare to bare electrode the modified electrode able to resolve the both ascorbic acid and uric acid in the solution mixture. The oxidation current of uric acid with the modified electrode is two folds higher than the bare glassy carbon electrode. Using differential pulse voltammetry method, the oxidation current is linear with the uric acid concentration in the range 30  $\mu$ M - 575  $\mu$ M with the detection limit of 5  $\mu$ M (S/N = 3). Moreover uric acid in real samples can be determined using nano poly cresol red without any pretreatment of samples giving satisfactory results.

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### INTRODUCTION

Uric acid (UA), is a key biomarker for the diagnosis of several diseases, which is the primary end product of purine metabolism. The decreasing and increasing concentration of UA lead to various diseases such as gout, Lesch-Nyhan syndrome, hyper uricemia, leukemia and so on [1-3] Therefore, the monitoring of UA in human blood and urine is very important for the prevention of

the mentioned and other similar diseases. The major problems is frequently encountered in the electrochemical detection compounds is the serious interference caused by co-existing compounds especially the serious interference of ascorbic acid (AA), which exist in body fluid in relative high concentration. The normal concentration of AA in human plasma and urine are 0.16 mg - 1.5 mg per ml and 10 mg - 60 mg in 24 hrs urine respectively.

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Moreover AA at the electrode surface often gives rise to a serious overlapping peak with uric acid [4-6]. The different analytical methods were used for the determination of UA such as high-performance liquid chromatography [7, 8], gas chromatography [9], capillary electrophoresis [10], chemiluminescence [11,12], ultraviolet-visible spectrophotometry [13, 14], fluorimetry [15] and electroanalytical methods [16, 17]. Among these methods, electro-analytical methods have been found to be reliable because of its high sensitivity, selectivity and reproducibility [18, 19]. Several modifier materials like polymers [20-25], biomaterials [26-31], photocatalytic [32-39] nanomaterials [40-45] have been used to overcome this problem. In recent years, substantial efforts have been devoted for the development of electrochemical sensors based on electrodes modified by electro-synthesized dye based nano polymeric films, due to their cost effective nature, high surface area, effective mass transport, and electrocatalytic [46, 47]. In this work, nano polymeric CR film was synthesized through electro polymerization method. The developed biosensors showed high sensitivity and reproducible in wide-linear range of potential. Further a anti-interference ability, long term stability and low detection limit were also examined for effective and selective determination of UA the presence of AA.

## MATERIALS AND METHODS

Uric acid, Ascorbic acid,  $[\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}]$ , 8-hydroxyquinoline, cresol red, 0.1M of sodium phosphate buffer, HCl, ethanol and all other chemicals are used of analytical grade. The solutions were prepared by using double distilled water. The experiments were carried out at 25 °C.

### Characteristic Studies

Cyclic Voltammetric (CV), Differential Pulse Voltammetric (DPV) experiments were performed using a CHI6041C electrochemical workstation (CH Inc., USA) coupled with a conventional three-electrode cell. The three electrodes namely the working electrode, the auxiliary electrode and the reference electrode were GCE, Pt wire and Ag/AgCl electrode respectively. All the potentials in this work are given against the Ag/AgCl. BRUKER RFS 27: Stand alone FT-Raman Spectrometer was used to find the functionalization of gold with 8-HQ, SEM (FEI Quanta FEG 200-High resolution scanning

electron microscope) was used to find the surface morphology. EI-1L model of 34 kHz ultrasonic bath was used for cleaning the electrodes and to prepare homogeneous mixture.

### Standardization of Glassy Carbon Electrode

Before modifying, the GC electrode was polished with 0.3 $\mu\text{m}$  and 0.05 $\mu\text{m}$  of alumina slurries for 2 min each step, followed by thoroughly rinsing with double distilled water, then the electrode was sonicated with ethanol and distilled water for 2 min each. After sonicating the electrode, it was rinsed with double distilled water and was examined by cyclic voltammetry using standard 1mM potassium ferric cyanide solution with 0.1M KCl as supporting electrolyte.

### Preparation Poly Cresol Red GCE

The 1mM cresol red was standardized with pH 6 phosphate buffer solution, then the three electrode setup was constructed, and the glassy carbon electrode was scanned up to 7 cycles between the potential of -0.7V to +1.6V with a scan rate 100  $\text{mVs}^{-1}$ . Now this electrode is said to be poly cresol red electrode, after this process the electrode was washed thoroughly with double distilled water to remove the physisorbed cresol red, then the modified electrode has to be immersed in 0.1M phosphate buffer solution of pH 7.0 until use.

## RESULTS AND DISCUSSION

### Surface Morphology Study & Electrodeposition of Poly Cresol Red polymerization

Fig. 1 shows cyclic voltammograms of 1mM CR in pH 6.0 phosphate buffer solution at GCE. In the first cycle, two strong anodic peaks were observed at 0.3V (Pa1) and -0.1V (Pa2), which might correspond to the oxidation of CR monomer. A cathodic peak (Pc3) also appeared at about -0.2V in the first cycle. In the subsequent cycles the larger peaks were observed upon continuous scanning, which was reflecting the continuous growth of the film. The possible electro polymerization mechanism was described as follows: Ortho-methyl phenol ring in CR was firstly oxidized to form 2-methylcyclohexadienone, and then a dimer was produced from a Michael addition reaction between the parent molecule and the oxidized form of CR (Fig. 2). This insoluble polymeric species formed by addition polymerization subsequently deposited onto the electrode.

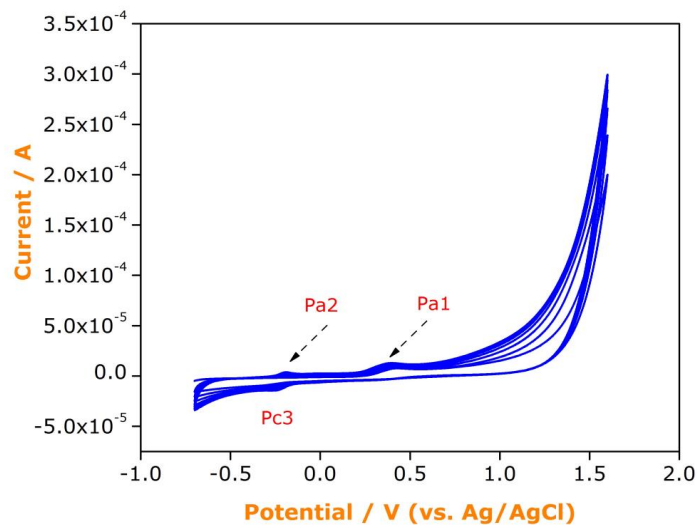


Fig. 1. Cyclic voltammogram of poly cresol red

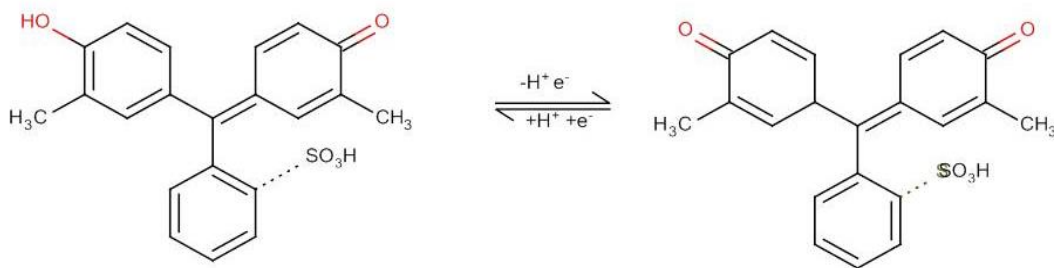


Fig. 2. Polymerization mechanism of Poly CR over the surface of GCE

These facts indicated that poly cresol red films were deposited on the surface of GC electrode by electro polymerization. The electrochemical behavior of poly cresol red at the GC electrode was in good agreement with previous findings, referring to the electrochemical responses of a few organic polymerized compounds at solid electrode [48]. Fig. 3(a-d) explains the surface morphology of the poly CR modified GCE using Field Emission Scanning Electron Microscopy (FESEM). The formation of small irregular spherical particles indicates that the glassy carbon has been modified by nano poly CR. It is noteworthy to mention that the entire surface area has been uniformly coated by the polymeric film.

#### Electrocatalytic Behavior of Poly Cresol Red GCE

The electrocatalytic behavior of poly cresol red was investigated with DPV in 0.1 M Phosphate Buffer Solution (PBS) at pH 7.0 as shown in Fig. 4. A weak response and slow electron transaction for both UA and AA is observed on a bare electrode

moreover the bare GCE unable to separate both AA and UA in the solution mixture, where as the nano poly CR modified electrode shows a good electrocatalytic oxidation toward both UA and AA (as seen in Fig. 4), indicating that the nano poly CR modified electrode can effectively separate both UA and AA in the solution mixture with decrease in the oxidation potential of UA to 0.26 V from a broad range of potentials. Furthermore, the oxidation peak current ( $I_{pa}$ ) of UA at the nano poly CR modified electrode is ten times higher than that of the bare electrode and for AA it is three times higher. These results confirm that the nano poly CR on the surface of the bare electrode can effectively accelerate the electrochemical redox behavior of UA and AA.

#### Effect of pH of UA in the Poly CR Modified GCE

The effect of pH on the peak current and peak potential of the catalytic oxidation of UA was investigated by cyclic voltammetry method. As a result shown in Fig. 5, the peak potential for UA oxidation

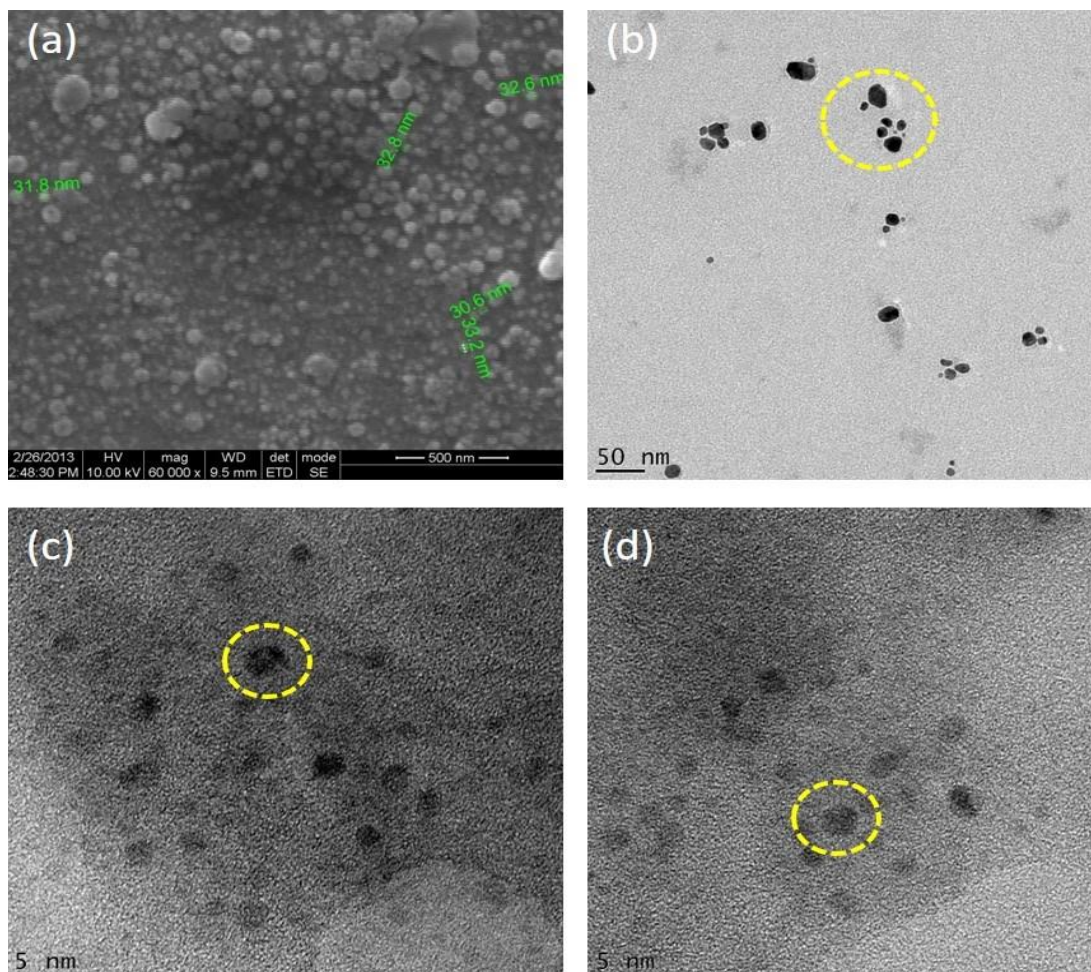


Fig. 3(a-d). FESEM image of nano poly CR modified surface

showed a linear variation with the increased value of pH and it shifted to a more negative potential with a slope of about  $-55 \text{ mV/pH}$ , which suggested that the total number of electrons and protons taking part in the oxidation of UA was the same. As the oxidation of UA is known to occur by a two-electron transfer [49], thus the number of protons involved is also predicted to be two (as shown in Fig. 6) Moreover oxidation of UA was carried out in broad range of pH ranging from 3-7. Since maximum peak current was observed in pH 7.0, this was taken for the further analysis Fig. 7 respectively.

*Effect of Scan Rate for UA in Poly Cresol Red GCE*

Effect of scan rate was studied by cyclic voltammetry method by placing 5mM UA in an electrochemical cell. The scan rate has been varied from  $50 \text{ mVs}^{-1}$  to  $300 \text{ mVs}^{-1}$ . Fig. 8 and Fig. 9 show anodic peak current of UA proportional with the square root of scan rate ( $v^{1/2}$ )

with correlation coefficient 0.9797. This observation suggests that oxidation process of UA in nano poly CR modified electrode followed diffusion by controlled process [50].

*Concentration UA in the Presence of AA in Poly Cresol Red GCE*

The DPV method is normally used for the determination of compounds because of its high sensitivity [51]. It is well known that AA is an electroactive molecule coexisting in a biological system, which can also be oxidized in the conventional solid electrode [52]. UA in the presence of AA by nano poly CR modified GCE is able to separate both 1 mM Uric acid and 1 mM AA in the potential range of  $-0.2 \text{ V}$ ,  $-0.8 \text{ V}$  vs Ag/AgCl Fig. 4. The DPV curves at different concentration of UA at the modified electrode clearly show that the anodic peak current increases linearly with an

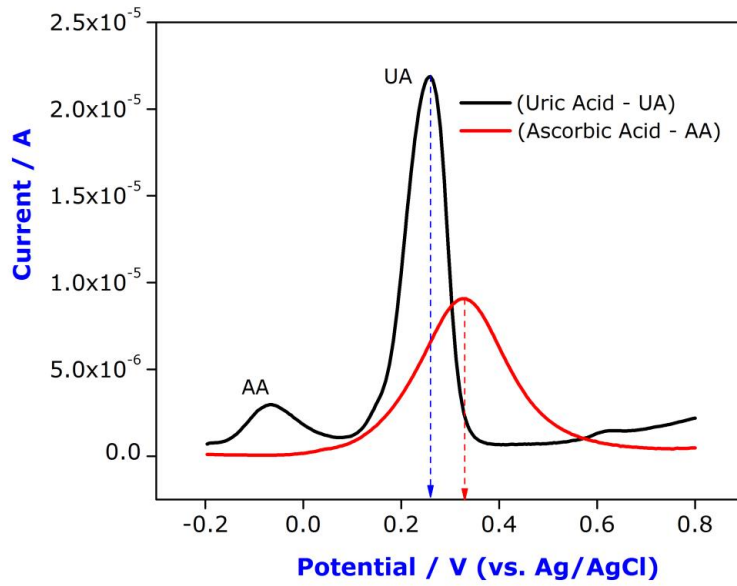


Fig. 4. DPV of 1mM UA and AA (a) bare GCE (b) nano poly CR GCE

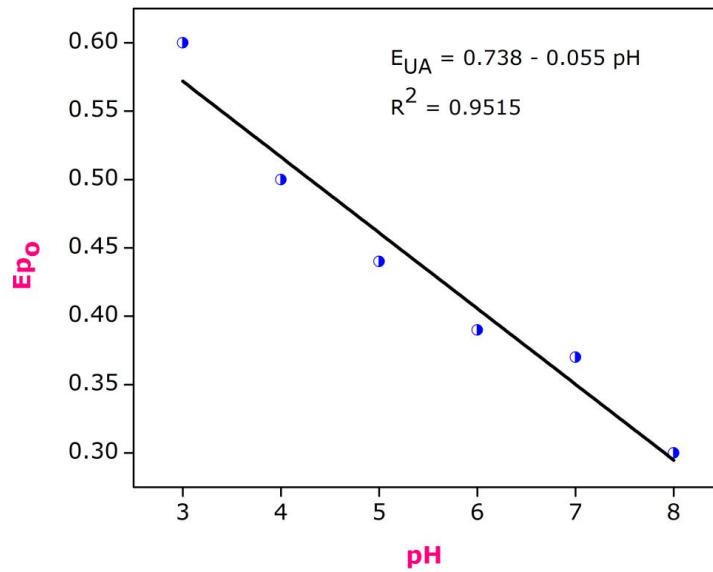


Fig. 5. Plot of  $E_{p0}$  (UA) Vs pH in poly CR GCE

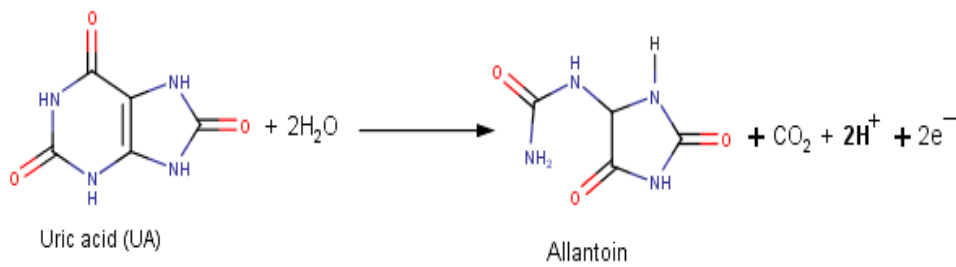


Fig. 6. Electro oxidation Mechanism of UA at nano poly CR modified GCE

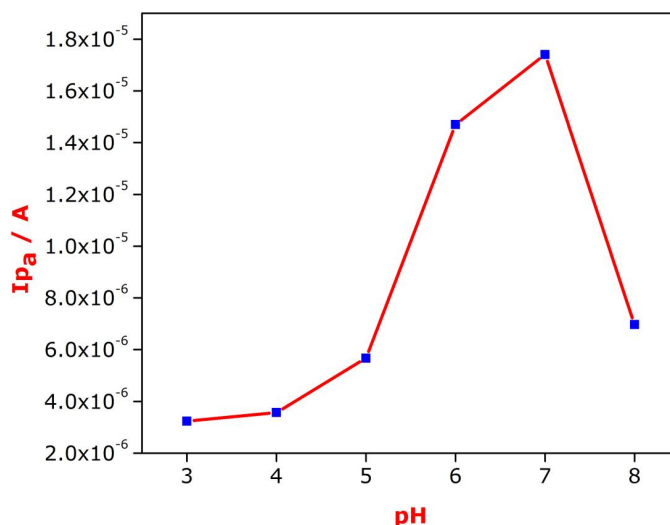


Fig. 7. Plot of ipa (UA) Vs pH in poly CR GCE

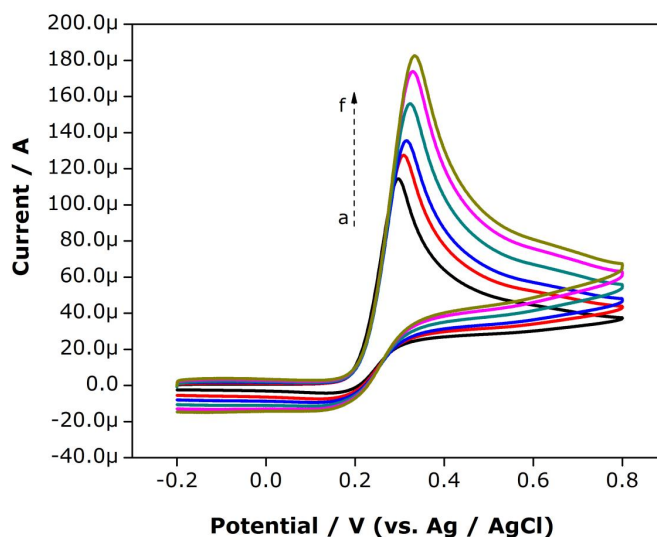


Fig. 8. Effect of Scan rate: cyclic voltammograms of UA (5mM) in the nano poly CR modified GCE in 0.1mol L<sup>-1</sup> PBS (pH-7.0) at various scan. The scan rate (from a - f) is 50, 100, 150, 200, 250, and 300 mV/s<sup>-1</sup>, respectively.

increase in UA concentration from 30 to 575  $\mu\text{M}$  Fig. 10. The correlation coefficient of the various concentrations of UA vs peak current is found to be 0.996 which is shown Fig. 11. Further the limit of detection is 5  $\mu\text{M}$  in the presence of 5 mM ascorbic acid. All this result illustrates that the coexistence of AA has no influence on UA determination.

#### *Interference of Coexisting Compounds of Uric acid in Real Samples*

A part from the presence of AA, the other reason for variation of UA oxidation peak current

is the presence of other interfering compounds. For this a 300  $\mu\text{M}$  of urea, 60  $\mu\text{M}$  of oxalate, 60  $\mu\text{M}$  of glucose were added and tested in the presence of 20  $\mu\text{M}$  UA. The percentage of change in anodic peak current is 5.6 %, 4.2 %, 4.5% for urea, oxalate and glucose respectively. This results show that the interfering compounds has no significant effect in the detection of UA. In comparing other interfering compound urea were tested in large amount due to its large excess amount in urine. The proposed method was validated by analysis with the real samples. For this the human urines

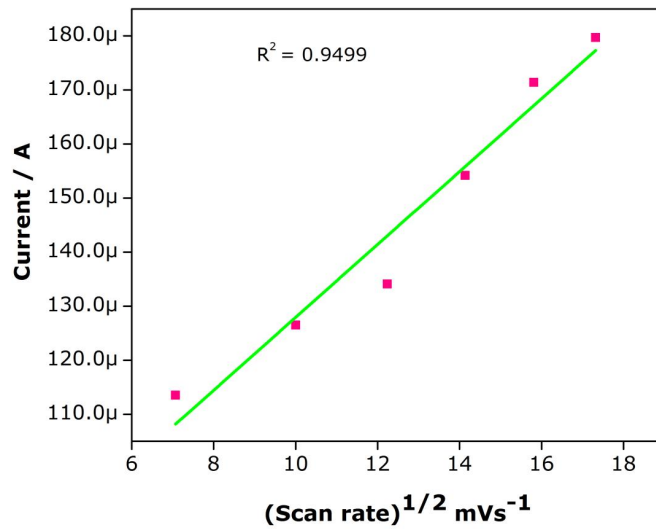


Fig. 9. Plots of ipa (UA) vs. square root of the scan rate in nano poly CR

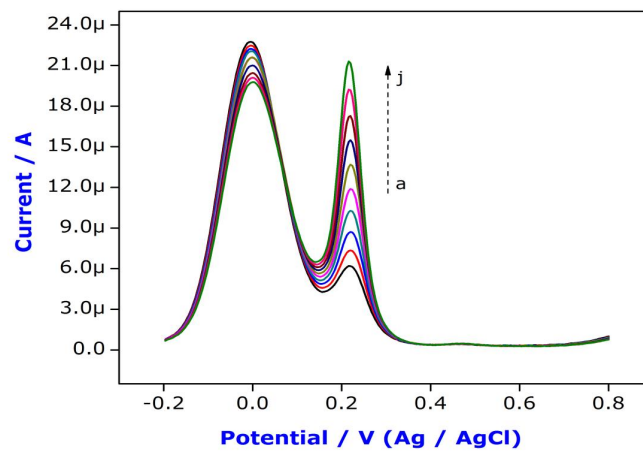


Fig. 10. DPV of AA (5mM) at nano structured poly CR electrode in the presence of different concentrations of L-dopa in μM: (a) 30 (b) 65 (c) 105 (d) 150 (e) 200 (f) 255 (g) 315 (h) 380 (i) 450 (j) 575

Table 1: Determination of UA in urine samples

S.No	Nature of sample	Original value (μM)	Spiked value (μM)	Found (μM)	Recovery (%)
1.	Urine	22.2	10	30.8	95.6%
2.	Urine	20.4	10	29.7	97.6%

were collected from the healthy volunteers. The human urine samples were filter and centrifuged before the experiment. Then the samples were diluted for 10 times with the phosphate buffer solution of pH 7.0. DPV was carried out with the diluted samples. The results are presented in the Table 1.

#### Stability and Reproducibility of the Nano Poly CR Modified GCE

The reproducibility of the developed sensor has been measured by repetitive scanning method in PBS pH 7.0. For 10 determination of UA at poly CR modified GCE the obtained relative standard deviation (R. S. D) is 4.8 % and 5.1 % this indicates

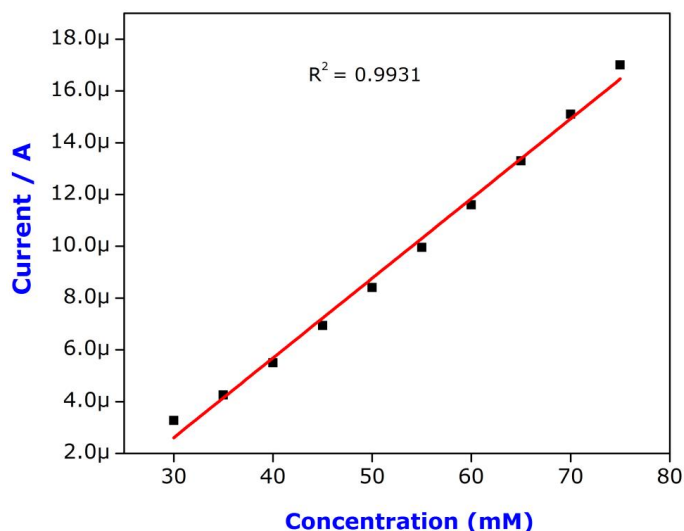


Fig. 11. Plot of UA ipa Vs Concentration of UA at nano poly CR modified GCE.

that the prepared polymeric film has excellent ability to prevent the electrode from the surface fouling. Further the long term stability of the prepared modified GCE has been studied. For the first 3 days there is no apparent current decrease was observed in every day use and stored in 0.1 M PBS (pH 7.0). after that 20 % of decreasing current was observed on every day use. This shows the presence of nano polymeric film over the surface of the GCE.

#### CONCLUSION

The present study has demonstrated the development of electrochemical detection of UA that are based on the electro polymerization of nano polymeric film. The surface morphology of the poly CR has been examined by using FESEM analysis. The results indicated that polymers were homogeneously deposited on the surface of the GCE with the particle size ranges from 30 nm - 65 nm. The electro polymerized nano poly CR shows good electro catalytic effect towards UA and AA in the solution mixture. Further it shows significant enlargement in peak current and a great decrease in the peak potential. The reproducibility as well as the selectivity is good. The selective detection of the UA in the presence of AA is accomplished with the detection limit of 5.0  $\mu$ M. Moreover the recovery percentage of 95.6 % and 97.6 % for UA determination in urine samples indicates that the developed sensor is very much reliable.

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#### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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