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Original scientific paper

Yellow DS5R polymeric film modified glassy carbon electrode for voltammetric assay of uric acid

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Abstract

This work focuses on the development of a novel and affordable yellow DS5R polymeric film on the glassy carbon electrode surface (Po-YDS5R/GCE) for the detection of uric acid (UAC). Po-YDS5R/GCE was effectively developed using the cyclic potentiodynamic polymerization technique. The sweep rate study revealed that the overall electrode reaction is diffusion-controlled. The linear range of 10-60 µM of UAC was used to compute the limit of quantification and limit of detection, and the results yielded 3.12 and 0.87 µM, respectively. The interference of dopamine on the electrooxidation of UAC was tested at the developed sensor. It was discovered that the modified sensor allows selective detection of UAC. These impressive outcomes showed that a simple and less costly Po-YDS5R/GCE sensor has the potential to make significant improvements over current electrochemical sensor technology.

Keywords
Reactive dye; electropolymerization; electroactive biomolecules; cyclic voltammetry

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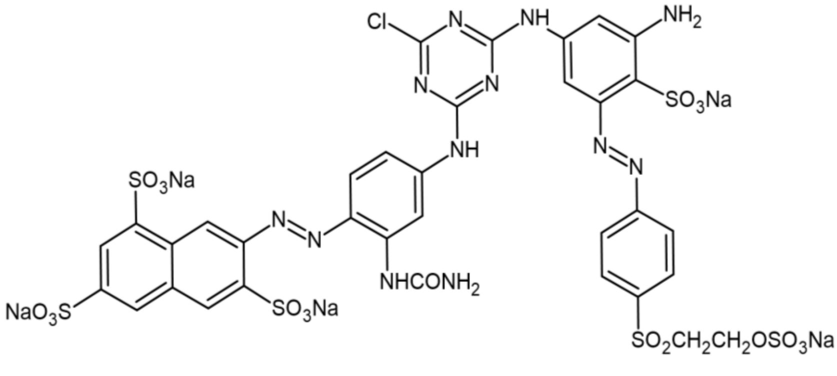
Introduction

Voltammetric methods are a crucial choice in drug analysis for detecting the active agent both qualitatively and quantitatively. The most effective voltammetric approaches offer great sensitivity, affordability, flexibility, time-saving, consistency, and an environmentally friendly nature [1-4]. These strategies have evolved in recent years, demonstrating that they constitute variable alternatives to other analytical methods.

Uric acid (UAC) is a by-product of the human body's purine nucleotide catabolism. The majority of UAC is dissolved in the blood and travels through the kidneys, which are excreted in urine [5-7]. UAC is an extremely biologically important molecule found in body fluids, forming the ions and salt referred to as urates and acid urates, including ammonium acid urate [8]. Massive content of blood UAC can induce hyperuricemia, which results in the development of UAC crystals and irreversible bone, joint, and tissue damage, as well as renal and heart illness. The crystals create urinary stones in the kidneys and gouty arthritis in the joints, which are the most prevalent and common associated disorders [9-13]. Several data revealed that increased blood UAC could be a substantial modifiable risk element [14]. As a response, UAC detection is crucial for reducing clinical consequences and preventing diseases that are linked to it. The frequent coexistence of UAC and dopamine (DA) body fluids in humans is well recognized. Due to their comparable oxidation potential, DA inferences the UAC detection [15,16]. Up to now, different modified electrodes have been designed to fix these issues.

In voltammetric methods, choosing an optional working electrode material is of the utmost importance. The glassy carbon electrode (GCE) is a practical conductive framework with a defined surface alignment, a large operating window, good mechanical properties, and ease of surface modification, all of which provide specific and selective results [17-20]. However, the naked GCE does not demonstrate substantial and selective separation of biomolecules in the presence of interferents [21-23]. Therefore, to get around this problem, electrochemical researchers have discovered electrode modification with unique conducting layers. There are a lot of prior literature articles on the modification of GCE by electropolymerization, producing materials like poly(neutral red), poly(4-amino-3-hydroxynaphthalene-1-sulfonic acid (AHNSA)), poly(aminobenzene sulphonic acid (ABSA)), poly(allura red), or poly(luminol) [24-28].

Here, we are concerned with the electropolymerization of Yellow DS5R (Scheme 1) polymeric film on the glassy carbon electrode surface by the potential cycling CV method. Such prepared Yellow DS5R polymeric film on the glassy carbon electrode surface (Po-YDS5R/GCE) was tested for the detection of UAC. By examining all outcomes of the CV technique, all experimental and voltammetric variables were checked. The objective of this work is to find a precise, ecologically safe, easy, and inexpensive approach for evaluating UAC.



Scheme 1. *The structure of Yellow DS5R dye*

Experimental

Chemicals, reagents and instruments

UAC, DA, NaOH, Na_2HPO_4, and NaH_2PO_4 were purchased from Nice Chemicals. Yellow DS5R was obtained from Astik Dyestuffs Pvt. Ltd. Gujarat. All analytical grade compounds were employed directly after delivery without any refining. 25 mM UAC was prepared in NaOH (0.1 M) solution, 25 mM DA in perchloric acid (0.1 M), and 25 mM Yellow DS5R in double distilled water. By mixing appropriate amounts of Na_2HPO_4 and NaH_2PO_4, a phosphate-borate buffer solution (PBS) of 0.2 M, pH 7.4 was made. Double-distilled water was used to prepare all solutions.

The electrochemical studies were conducted using voltammetric equipment of model CHI-660C (CHI-660 electrochemical workstation). The three-electrode cell system consisted of glassy carbon (GCE) and Po-YDS5R/GCE working electrodes, a saturated calomel reference electrode (SCE) and a platinum counter electrode. At all CV recordings, the initial potential scan was performed in a positive direction, denoted by arrows in all graphs.

Configuring GCE and Po-YDS5R/GCE

Alumina powder was used to polish the GCE before cleaning with de-ionized water. The GCE was successfully modified by electropolymerization from 1.0 mM aqueous solution of Yellow DS5R in 0.1 M NaOH as a supporting electrolyte. As illustrated in Figure 1a, the potential range was sustained between -0.6 and 1.6 V *vs*. SCE at a sweep rate (SR) of 100 mV/s for 10 cycles.

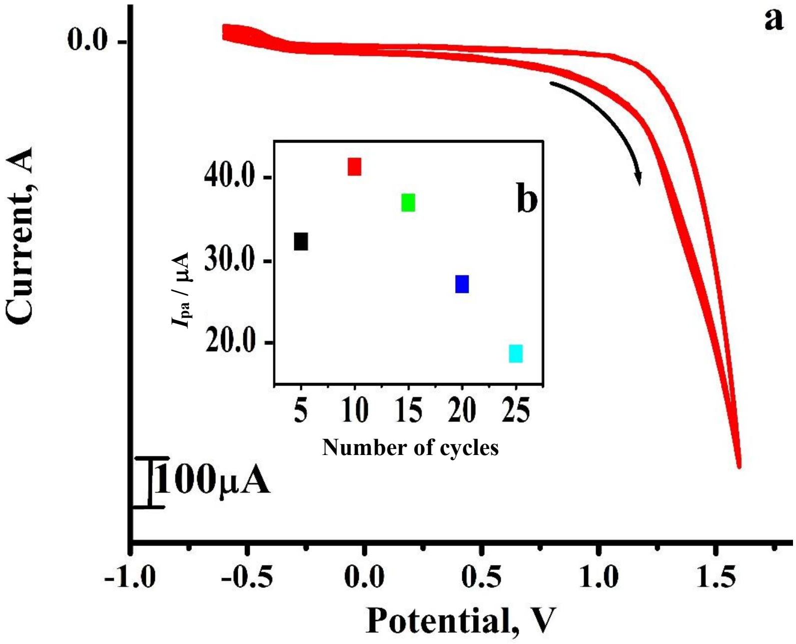


Figure 1. *a) CVs of fabrication of Po-YDS5R/GCE from1.0 mM Yellow DS5R in 0.1 M NaOH at 10 sweep cycles with sweep rate 100 mV/s; b) linearity plot of anodic peak current* I_{pa}, vs. *number of cycles*

Results and discussion

Optimization and electrochemical characterization of Po-YDS5R/GCE

The electrode coating by the polymeric material affects the electrochemical reactivity of the modified electrode. It was simple to adjust the coating thickness during electropolymerization of 1.0 mM aqueous solution of Yellow DS5R in 0.1 M NaOH as supporting electrolyte on the GCE by increasing the number of sweep cycles from 5 to 25. The voltammogram was first gradually increased by increasing the sweep cycles due to the formation and development of the electroactive Yellow DS5R layer on the GCE surface. After a few further sweeps, however, the electrocatalytic ability of the Po-YDS5R/GCE decreased as the polymer film thickness increased, preventing the electron transfer process (inset Figure 1b) [29]. As a result, it was determined that 10 sweep cycles are ideal for obtaining the potent electrocatalytic response of the dye polymer layer.

Figure 2 demonstrates CVs for the $K_4[Fe(CN)_6]$ system in 1 M KCl (supporting electrolyte) at GCE (line a) and Po-YDS5R/GCE (line b) with a sweep rate of 100 mV/s. The tiny peak current was detected on the GCE, which is hugely enhanced for the Po-YDS5R/GCE, indicating a significant increase in the rate of electron transfer [30]. The electroactive area of the GCE and Po-YDS5R/GCE was computed using Randles-Ševčik equation (1) [31]:

$$I_p = 2.69\text{x}10^5 n^{3/2} D_0^{1/2} \nu^{1/2} AC \tag{1}$$

where I_p / A is peak current, A / cm^2 is the active surface area of the electrode, n denotes the number of electrons transferred, D / cm^2 s^{-1} is the diffusion coefficient of electroactive species, ν / V s^{-1} signifies the sweep rate and C / mol cm^{-3} designates the concentration of electroactive species.

Using Equation (1) and Figure 2, the active area was found to be 0.029 cm^2 for GCE and 0.039 cm^2 for Po-YDS5R/GCE, respectively.

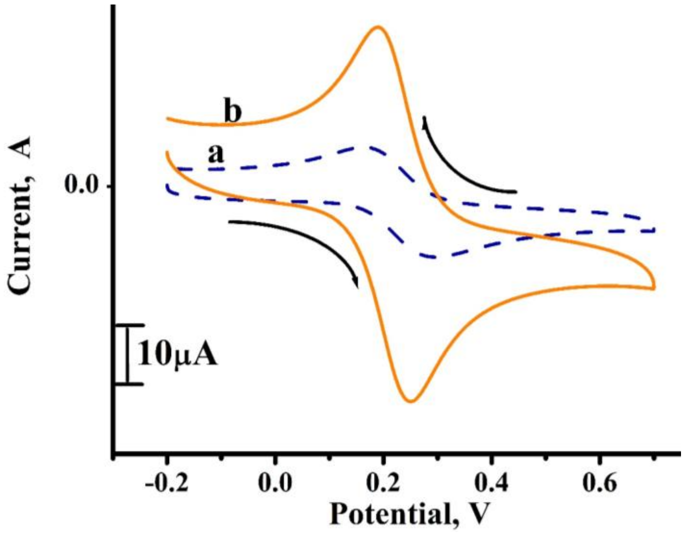


Figure 2. *CVs at 100 mV/s of 1 mM $K_4[Fe(CN)_6]$ in 0.1 M KCl at GCE (line a) and Po-YDS5R/GCE (line b)*

Voltammetric analysis of UAC at Po-YDS5R/GCE

Figure 3 depicts the CVs for electrochemical oxidation of 0.1 mM UAC at GCE (line a) and Po-YDS5R/GCE (line b) in PBS (0.2 M, pH 7.4) with SR of 100 mV/s. The UAC displays a lower current at 0.280 V with a broad peak at GCE, which suggests slow electron transfer kinetics because of fouling of the electrode surface as a result of the oxidation process. Po-YDS5R/GCE, however, shows a higher peak current at 0.266 V and a minimal shift in the potential. The rise in peak current is due to the fast electron transfer kinetics of UAC at the Po-YDS5R/GCE surface.

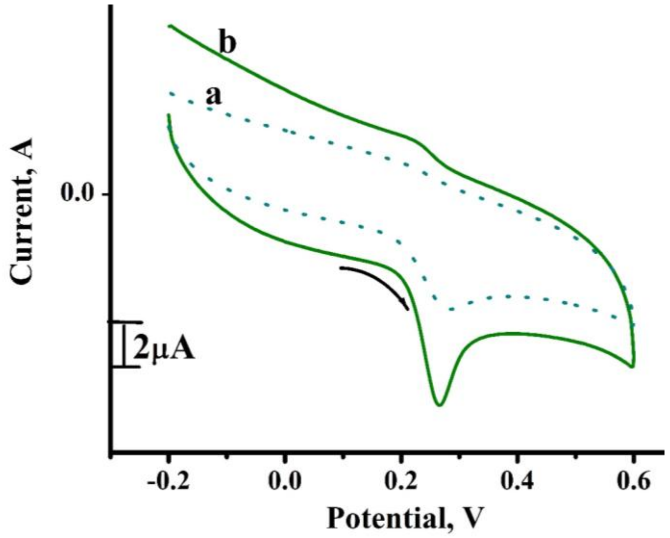


Figure 3. *CVs at 100 mV/s of 0.1 mM UAC in PBS (0.2 M, pH 7.4) at GCE (line a) and Po-YDS5R/GCE (line b)*

Sweep rate and pH impacts

Figure 4a details the CVs results for 0.1 mM UAC in PBS (0.2 M, pH 7.4) on Po-YDS5R/GCE at varied sweep rates from 50-500 mV/s. The raised electrode oxidation peak current and small positive shift in the oxidation potential is noticed when raising the sweep rate 50-500 mV/s, which agrees with Randles-Ševčik's relationship (equation 1). Figures 4b and 4c show good linearity of plots presenting anodic peak current (I_{pa}) *vs*. sweep rate and I_{pa} *vs*. square root of sweep rate. The procured outcomes were fitted to the following linear relations: $I_{pa} = 47.0\nu + 5.70$ ($R^2 = 0.996$) and $I_{pa} = 11.4\ \nu^{1/2} + 2.07$ ($R^2 = 0.996$), which reveals that the oxidation reaction of UAC at Po-YDS5R/GCE surface is diffusion controlled [32].

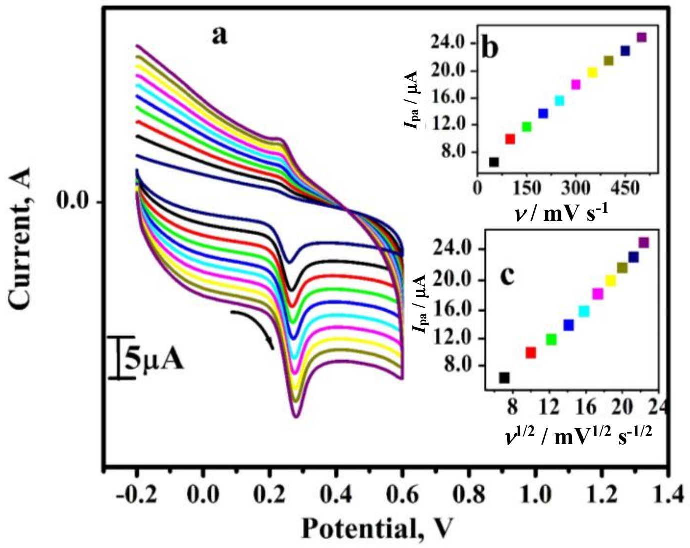


Figure 4. *a) CVs of 0.1 mM UAC in PBS (0.2 M, pH 7.4) at Po-YDS5R/GCE at varied sweep rate of 50 to 500 mV/s; b) plot of* I_{pa} vs. *sweep rate; c) plot of* I_{pa} vs. *square root of sweep rate*

For pH optimization, buffer solutions containing 0.1 mM UAC were made at a pH of 6.2 to 7.8. Figure 5a shows the CV responses at varied pH, clarifying that all peak potentials (E_{pa}) are shifted towards negative potential as pH increases, according to the Nernst formula [34]. The linearity graph of E_{pa} *vs*. pH (Figure 5b) was fitted with the linear regression equation, $E_{pa} = -0.071\ \text{pH} + 0.79$ ($R^2 = 0.999$), which confirms that the same quantity of protons and electrons are participating in the process (Scheme 2) [33,34].

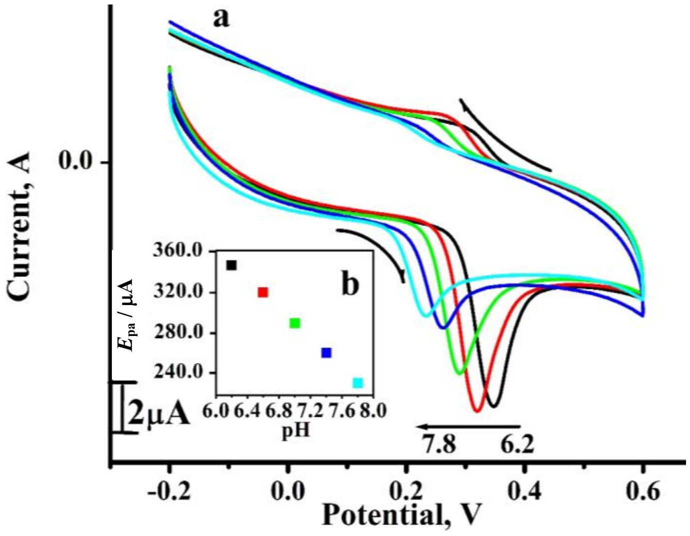
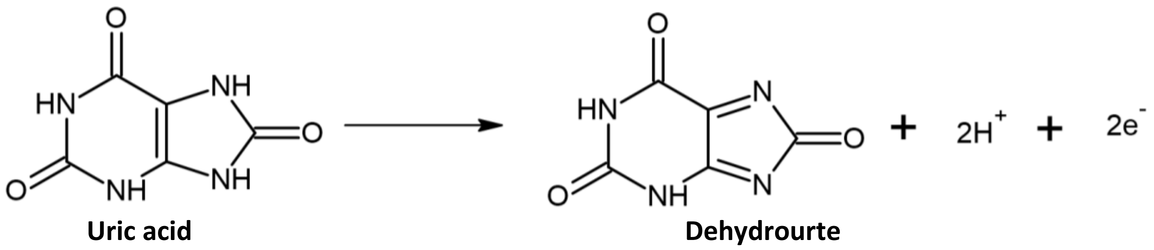


Figure 5. *a) CVs responses for 0.1 mM UAC at Po-YDS5R/GCE in 0.2 M PBS of different pH (6.2 to7.8); b) plot of* E_{pa} vs. *pH*



***Scheme 2.** Electrooxidation of UAC*

Assay of UAC at varied concentration

Po-YDS5R/GCE was employed to assay UAC in a range of different UAC concentrations (10 to 60 mM) in PBS buffer (0.2 M, pH 7.4) by CV method. As seen in Figure 6a, CV curves gradually grew as the UAC concentration increased. When I_{pa} was plotted *vs.* UAC concentrations (Figure 6b), the regression expression I_{pa} / A = 3.18 C / M + 4.09 (R^2 = 0.999) shows good linearity. The limit of detection and limit of quantification were detected to be 0.87 and 3.12 µM. The Po-YDS5R/GCE's achievement has been compared to others previously reported and depicted in Table 1.

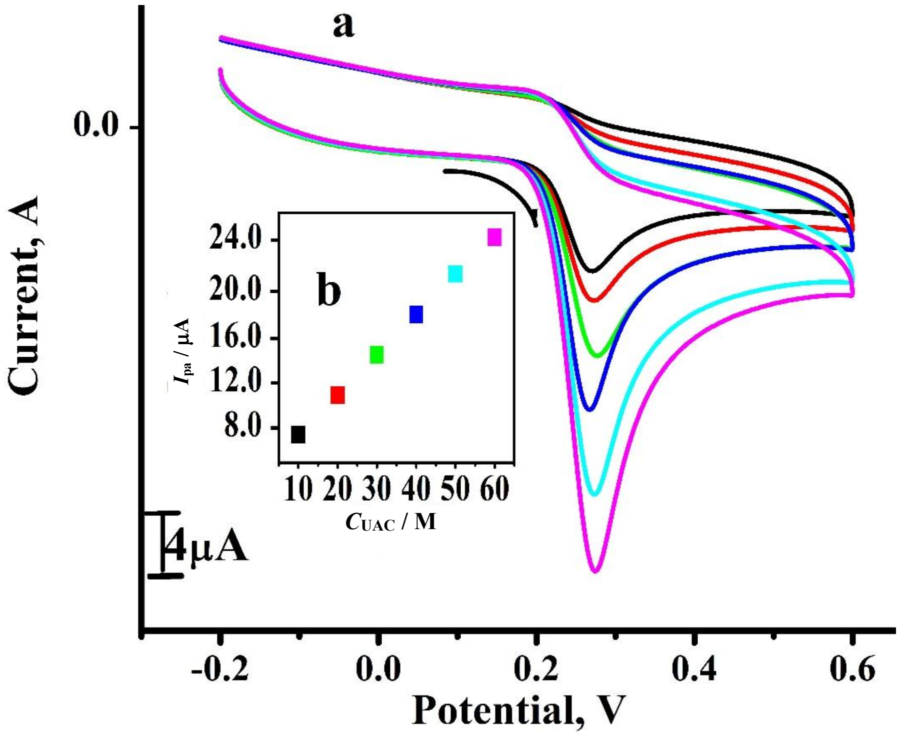


***Figure 6.** a) CVs at 100 mV/s of varied UAC concentrations (10 to 60 µM) at Po-YDS5R in 0.2 M PBS, pH 7.4; b) plot of* I_{pa} vs. *UAC concentration*

***Table 1.** Comparison of LOD values for UAC at Po-YDS5R/GCE with previous literature reports*

Electrode	LOD of UAC, µM	Method	References
Poly (o-aminophenol)-MCPE	3.0	CV	[35]
GA/GO/MCPE	4.18	CV	[36]
Pd/RGO/GCE	1.6	DPV	[37]
Glu/GCE	1.1	DPV	[38]
Au-Cu$_2$O/rGO/GCE	6.5	DPV	[39]
Pdop@GR/MWCNTs	15.0	DPV	[40]
PG/GCE	4.82	CV	[41]
Po-YDS5R	0.87	CV	Present paper

GA: glibenclamide; GO: graphene oxide; RGO and rGO: reduced graphene oxide; Glu: glutamic acid; Pdop@GR: polydopamine coated graphene PG: pristine graphene

Simultaneous study of UAC and DA

Figure 7 shows CVs of 0.1 mM UAC and 0.1 mM DA mixture in PBS (0.2 M, pH 7.4) at a sweep rate of 100 mV/s at GCE (line a) and Po-YDS5R/GCE (line b). At GCE, the obtained CV curve was broad and slightly sensitive to the oxidation of the analytes UAC and DA. The use of Po-YDS5R/GCE results in two discrete oxidation peaks at E of 0.299 and 0.128 V for UAC and DA, respectively, with a ramp-up in peak current. The electrolytic peak-to-peak splitting between UAC and DA was 0.171 V. These findings imply that UAC can be selectively assayed in the vicinity of DA at Po-YDS5R/GCE.

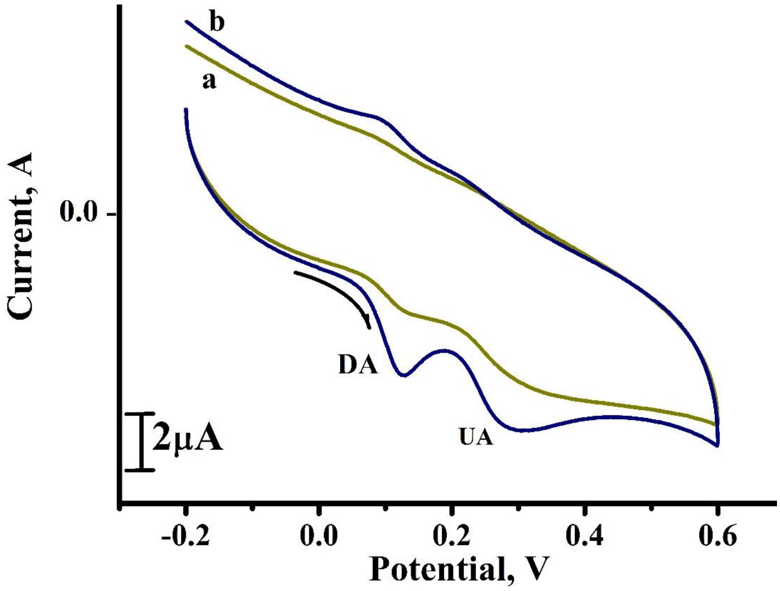


Figure 7. *CVs at 100 mV/s of simultaneous analysis of 0.1 mM UAC and 0.1 mM DA in PBS (0.2 M, pH 7.4) at GCE (line a) and Po-YDS5R/GCE (line b)*

Interference analysis

Interference analysis was done by combining UAC and DA in PBS (0.2 M, pH 7.4) at Po-YDS5R/GCE using the differential pulse voltammetry (DPV) technique. Figure 8 shows DPV responses of DA by boosting its concentration (10 - 60 μM) while holding the UAC concentration (10 μM) constant. It can be noticed in Figure 8 that only I_{pa} of DA increased significantly with increasing DA concentration.

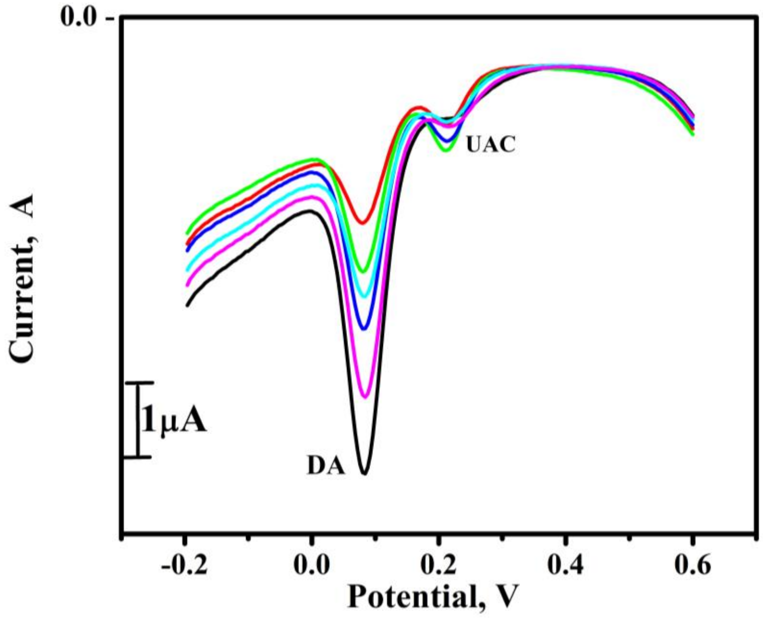


Figure 8. *DPV responses at 100 mV/s for varied concentrations of DA (10-60 μM) in the presence of UAC (10 μM) in PBS (0.2 M, pH 7.4) at Po-YDS5R/GCE*

On the other side, Figure 9 depicts the DPV responses of Po-YDS5R/GCE when the concentration of UAC was increased (10 to 70 µM) while DA concentration (10 µM) kept constant. Figure 9 clearly shows the enhancement of only I_{pa} of UAC in that case. The outcomes suggest that interference of DA did not affect the electrolytic activity of UAC. Therefore, the forecast method and Po-YDS5R/GCE are substantially free of interferences.

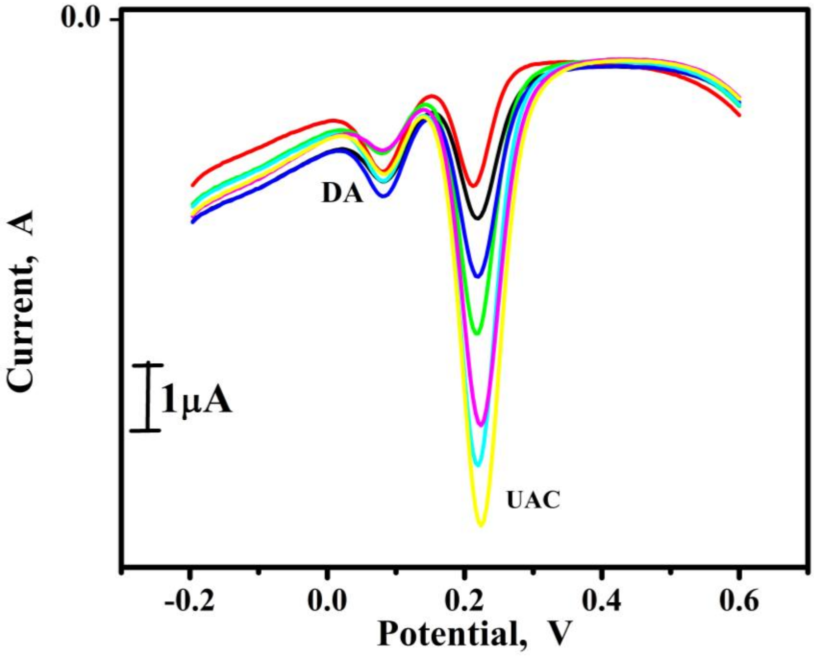


Figure 9. *DPV responses at 100 mV/s for varied concentration of UAC (10-70 µM) GCE in presence of DA (10 µM) in PBS (0.2 M, pH 7.4) at Po-YDS5R/GCE*

Stability and reproducibility

The stability of Po-YDS5R/GCE was tested by sweeping it for 20 cycles for 1 mM $K_4[Fe(CN)_6]$ (data not shown). The I_{pa} signals recovered at about 97.6 % of the initial value. Because of these results, Po-YDS5R/GCE has a lifespan and exceptional stability. The reproducibility of Po-YDS5R/GCE was examined after 5 days when kept at room temperature. The I_{pa} signal recollected 97.9 % of the earlier start current response and did not influence the peak potential.

Analytical application

To verify the analytical applicability of Po-YDS5R/GCE for UAC assay, some UAC concentrations were added to the human urine sample. The recovery values of UAC in the range of 96.2±4 % to 98.1±2 % for UAC concentrations in the range of 10 to 30 µM were projected using the standard addition method (Table 2). The values obtained were revealed to be acceptable.

Table 2. *Determination of UAC in human urine samples*

UAC concentration, µM		Recovery, %
Added	Estimated	
10	9.8	98.1±2
20	19.6	96.2±4
30	29.2	97.4±3

Conclusion

We have proposed a simple technique of electropolymerization for producing a reactive dye polymer, Po-YDS5R film, at GCE *via* the CV method. The resulting Po-YDS5R/GCE was employed for the assay of UAC. The key attractive properties of Po-YDS5R/GCE are the swift modification process, increased sensitivity, high selectivity, low cost, and sustainability. The LOD of 0.87 µM was achieved at the developed sensor with the merit of interference-free determination. The fabricated Po-YDS5R/GCE delivered good recovery in the human urine sample. As a result, the Po-YDS5R/GCE sensor will hold high potential in biosensors, where it can be employed to determine other electroactive biomolecules.

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