

## Nile Blue-Chitosan Composite as a New Electrochemical Sensor for Uric Acid on Glassy Carbon Electrode

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### ABSTRACT

**N**ile blue-chitosan as a new type of organic dye-organic composite was made using Nile blue and chitosan. The Nile Blue Chitosan composite was made using a 0.005 M solution of Nile blue and a 7% solution of chitosan. To do this, a mixture of Nile blue and Chitosan with the ratio of 2:1 was prepared. The electrocatalytic oxidation of uric acid has been studied on glassy carbon electrode by Nile Blue Chitosan using electrochemical techniques. The Nile blue-chitosan electrode showed one pair of peaks in  $KHSO_4$  (0.5 M) as a supporting electrolyte. The catalytic currents are linearly related to UA concentrations in the ranges of  $3 \times 10^{-5}$  to  $1.8 \times 10^{-4}$  M and limit of detection  $1.2 \times 10^{-6}$  M with surface coverage of  $2 \times 10^{-8}$  mol  $cm^{-2}$ . Prog. Color Colorants Coat. 8(2015), 1-9 © Institute for Color Science and Technology.

### 1. Introduction

Nile blue (NB) was introduced by Lorrain Smith (1908) for distinguishing neutral fats (triglycerides) from fatty acids, the former being red and the latter dark blue [1]. Since that time NB was used in many applications. NB is a basic cationic phenoxazine dye which is soluble in water and ethyl alcohol. Its planar hydrophobic phenoxazine structure is expected to facilitate the intercalation of NB with the relatively non-planar interior of the DNA helix [2]. Also, its amine and imine moieties facilitate its interactions with natural polymers (Figure 1).

Chitosan is a linear polysaccharide composed of

randomly distributed  $\beta$ -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating shrimp and other crustacean shells with the sodium hydroxide. Chitosan has a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and bio-pesticide, helping plants to fight off fungal infections [3]. It contains more than 5000 glucosamine units and is obtained commercially from shrimp and crab shell chitin (a N-acetylglucosamine polymer) by alkaline deacetylation (NaOH, 40-50%) [4].

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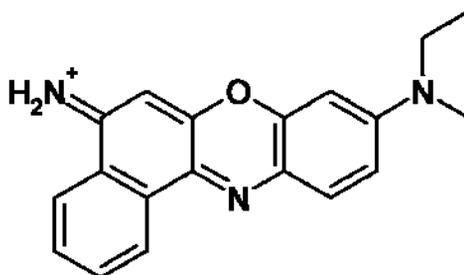


Figure 1. The structural formula of Nile blue.

Recent advances in molecular biological techniques suggest that chitin deacetylation can be done by enzymes with novel properties that can be used in preparation of chitosan polymers and oligomers [4]. Chitosan is insoluble in most solvents but is soluble in dilute organic acids such as acetic acid, formic acid, succinic acid, lactic acid, and maleic acid. The use of chitosan is limited because of its solubility in water, high viscosity, and tendency to coagulate with proteins at high pH values. Many efforts to prepare functional derivatives by chemical modifications to increase the solubility in water have been reported [5, 6].

Applications for chitosan currently are found in industrial wastewater treatment [7] and recovery of food grade material from food processing plants [8]. Numerous studies have been conducted on the adsorption (or absorption) of metal cations such as lead, cadmium, copper, and nickel and the adsorption (or absorption) of oxyanions was achieved by using complexed metal ions [9, 10]. Extraction of dyes from waste water in an economic fashion for the textile industry is another important application of Chitosan [11].

NB can be adsorbed strongly on the electrode surface and is usually used for chemically modified electrode [12, 13]. NB is a phenoxazine dye that has shown electrocatalytic properties as a redox catalyst. Goroton group has extensively investigated the electrochemical properties of adsorbed NB on different electrode materials, such as graphite, glassy carbon and zirconium phosphate for electrocatalytic NADH oxidation [14]. Some scientists have extensively investigated the electrochemical properties of adsorbed NB on the electrodes of different materials as electrochemical sensors [15-17]. In this work, we used Chitosan as an adsorbent for NB and the

electrochemical and electrocatalytic behavior of the Nile Blue Chitosan (NB-CH) modified glassy carbon electrode is studied. To the best of our knowledge, there is no report on the preparation of NB-CH and its electrocatalytic properties.

## 2. Experimental

### 2.1. Chemicals and instrumentation

NB, chitosan and other chemicals were of analytical grade from Merck without further purification. The buffer solution was prepared using  $\text{H}_2\text{SO}_4$  (0.5 M) and the pH was adjusted by KOH not to exceed 7. Uric acid solutions were prepared just prior to use and all experiments were carried out at ambient temperature. All solutions were prepared with distilled water. Electrochemical techniques such as CV measurement were performed using  $\mu$ -Autolab PGSTAT Potentiostat/Galvanostat equipped with a frequency response analyzer (FRA4.9) and controlled by General Purpose Electrochemical System (GPES4.9) software (Eco Chemie, Utrecht, The Netherlands). The cell used was equipped with a modified glassy carbon as the working electrode. A platinum wire electrode was used as auxiliary electrode and Ag/AgCl as a reference electrode (Azar electrode Co. Iran). The pH was measured with a Zag Chemie model PTR79 pH/mV meter

### 2.2. Procedures

#### 2.2.1. Working electrode Fabrication

The glassy carbon (GC) electrode surface was polished with 0.05  $\mu\text{m}$  alumina powder on a wet polishing cloth. The polished electrode was rinsed with distilled water several times. The modified electrode was preparation as follows. The 7% (w/v) chitosan solution was

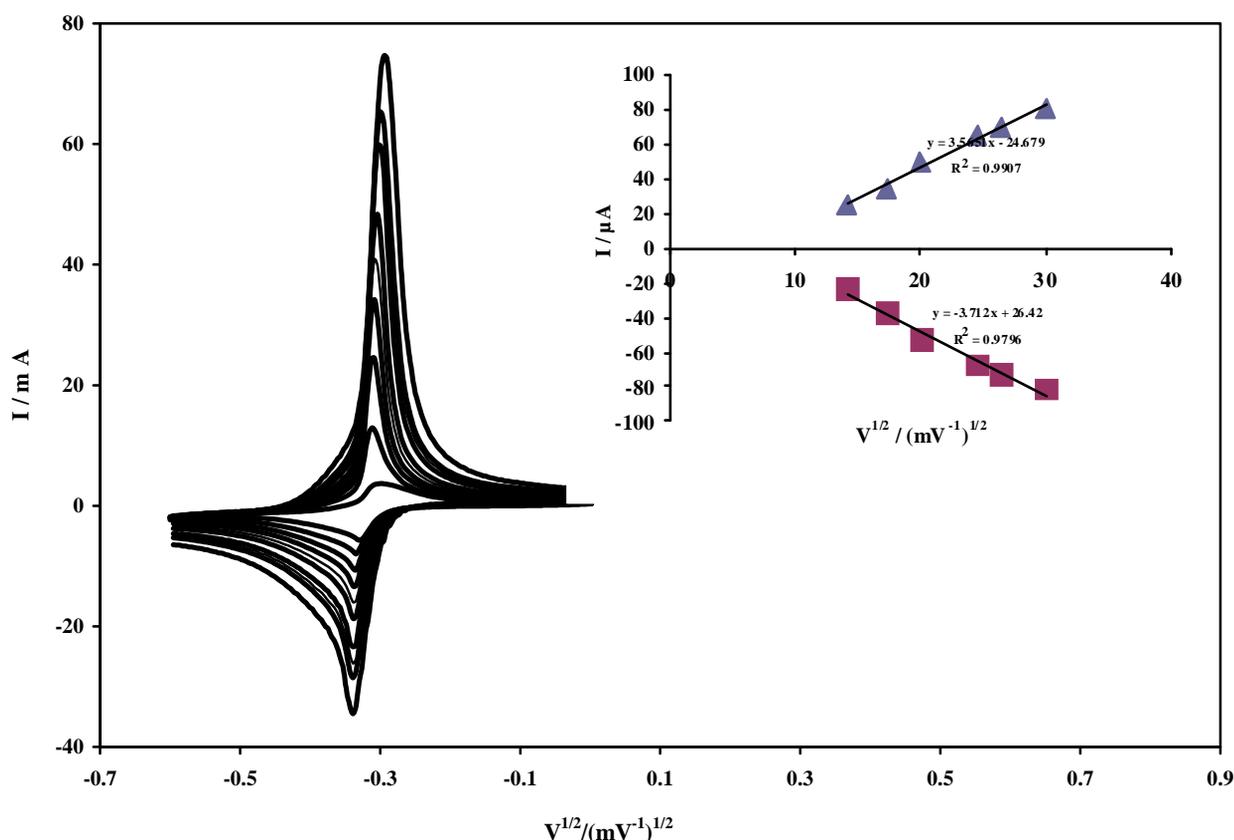
prepared in acetic acid (0.1 M) and was mixed with NB solution (0.005 M) by the ratio of 1:2. Then the mixture was stirred for 5 h to obtain a gel. NB-CH gel was placed directly on the glassy carbon electrode and let to dry for 2 h in room temperature. For electrochemical studies, the cyclic voltammetry was accomplished by cycling the electrode potential in the range of -0.6 V to 0.1 V vs. Ag/AgCl.

### 3. Results and discussion

#### 3.1. Electrochemical Characteristics of NB-CH GC electrode

In this study, NB-CH GC electrode prepared under the optimum experimental conditions (pH=7, 7% (W/V) chitosan in acetic acid (0.1 M), NB (0.005 M), 5 h mixing time, 2 h drying time) was characterized by cyclic voltammetry. The cyclic voltammograms of NB-CH modified glassy carbon electrode in the presence of 0.5 M sulphate buffer with pH 7 were recorded in the range of -0.6 V to 0.1 V vs. Ag/AgCl at various scan

rates 20-900  $\text{mVs}^{-1}$  (Figure. 2). As seen, the voltammograms show a pair of peaks. The anodic and cathodic potential peaks were -0.31 V and -0.34 V vs. Ag/AgCl, respectively. The formal potential, defined as the average of the anodic and cathodic peak potential,  $E^{\circ} = (E_{\text{pa}} + E_{\text{pc}}) / 2$ , is about -325 mV vs. Ag/AgCl and is almost independent of potential scan rate for sweep rates ranging from 20 to 100  $\text{mV s}^{-1}$ . The  $I_{\text{pa}} / I_{\text{pc}}$  ratio remains almost unit and peak separation,  $\Delta E_{\text{p}} = E_{\text{pa}} - E_{\text{pc}}$ , of 30 mV at a scan rate of 50  $\text{mV s}^{-1}$  was found. At higher scan rates, wider splitting appears, indicating the limitation arising from charge transfer kinetics. The peak currents of the voltammogram are linearly proportional to the scan rate up to 200  $\text{mV s}^{-1}$ , which is expected for surface confined redox process. For scan rates higher than 200  $\text{mV s}^{-1}$ , the anodic and cathodic peak currents are proportional to the square root of the scan rate which is expected for a diffusion-controlled electrode process (the inset in Figure 2).



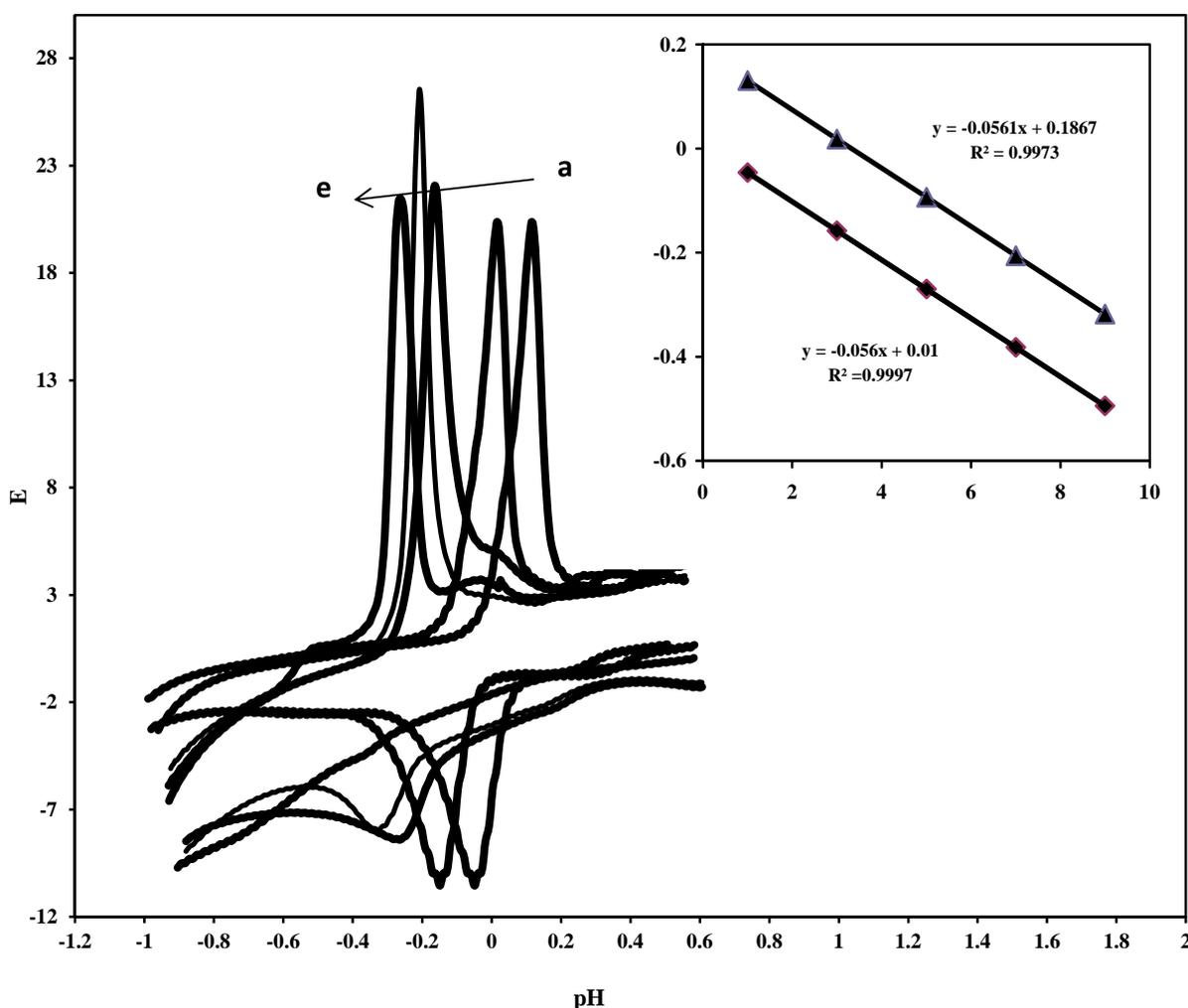
**Figure 2:** Typical cyclic voltammograms of NB-CH GC electrode and the plot of the peak current vs. Square root of scan rate (from inner to outer): 20, 50, 100, 200, 300, 400, 600, 700, 800 and 900  $\text{mV s}^{-1}$ .

### 3.2. Stability of NB-CH GC electrode

The stability of the NB-CH GC electrode was examined by repetitive scans in a 0.5 sulfate buffer solution. At continuous potential scanning, the peak current decreases for a few first scans then, negligible variation in height and separation of cyclic voltammometric peaks was observed. The decrease in NB-CH GC electrode response after 20 cycles was negligible. Furthermore, there is no loss of redox activity after storing modified electrode in air for a month.

### 3.3. Effect of pH

Cyclic voltammetry as an electrochemical technique was also used to study the effect of sulfate buffer pH on the peak currents. The resulting  $I_{pa}$  vs pH (1-9) data is illustrated in Figure 3. As can be seen, the peak potentials decrease with increasing pH up to 9. At higher pH values, the  $E_{pa}$  and  $E_{pc}$  shift toward more negative potential values. The plot of  $E_{pa}$  and  $E_{pc}$  versus pH shows a linear relation in the pH range of 1–9 with a slope of  $-56 \text{ mV pH}^{-1}$ , this is a Nernstian slope and it seems that this electrode shows a Nernstian behavior in pH range of 1-9 (the inset in Figure 3).



**Figure 3:** Cyclic voltammograms for NB-CH GC electrode in electrolytes with different pH values: (a) 1.0; (b) 3.0; (c) 5.0; (d) 7.0; (e) 9.0. Inset: plots of  $E_{pa}$ ,  $E_{pc}$  vs. pH.

### 3.4. Surface coverage of NB-CH GC electrode

The surface coverage of the NB-CH GC electrode,  $\Gamma$ , represent the moles of electroactive NB-CH redox sites per unite area of electrode surface. The amount of charge,  $Q$ , consumed for anodic peak recorded at  $20 \text{ mVs}^{-1}$ , was measured with respect to the base line and used to calculate  $\Gamma$  from equation (1):

$$\Gamma = Q/nFA \quad (1)$$

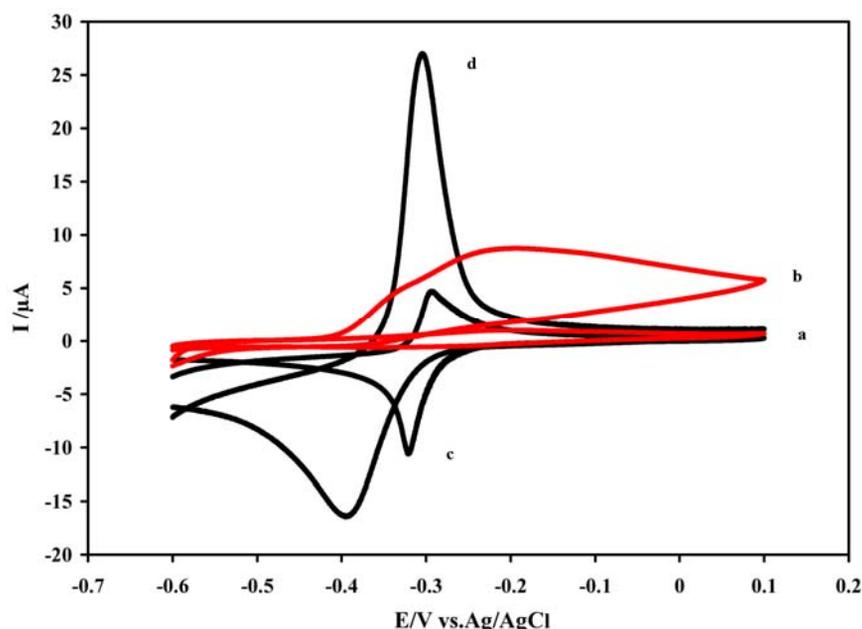
where  $n$ ,  $F$ ,  $A$  and  $\Gamma$  are the number of electron transferred in the surface redox reaction, the Faraday constant, electrode surface area and electrode surface coverage, respectively. It should be pointed out that the calculated surface coverage is an efficient attribute (per cross section of the electrode) and does not reflect the actual amount of NB-CH per area of the electrode. An increase of scan rate from  $20$  to  $900 \text{ mV s}^{-1}$  causes a continuous decrease of the apparent coverage from  $2.0 \times 10^{-8}$  to  $3.8 \times 10^{-10} \text{ mol cm}^{-2}$ . A decrease of the apparent coverage with increasing the scan rate is probably related to the charge transfers through modified electrode layer, which become rate limiting at higher scan rates. It is to say that at low scan rates, the time windows is long, while in high scan rates, time windows is short; this causes the surface coverage in

low scan rate to become larger than that of high scan rates.

### 3.5. Electrocatalytic response of NB-CH electrode to uric acid

Figure 4 shows the cyclic voltammetric behavior of the NB-CH electrode toward Uric acid (UA). There is a pair of reversible anodic and cathodic peaks. When  $0.5 \text{ mM}$  uric acid was added into the solution, cathodic peak current increased significantly. By increasing the oxidation peak current, the reduction peak current is also increased and NB-CH acts as a mediator in electrocatalysis of UA. It was observed that the cathodic peak-potential shifted toward negative values with the increase of uric acid concentration.

As can be seen in Figures 4 (curve b and d), once UA was added on GC and GC-NB electrodes, an increase in the anodic peak current was observed. This behavior is typical for electrocatalysis, but under same conditions the bare GC electrode showed a peak with low current (curve b). Figure 3 shows that the GC-NB electrode decreased the oxidation potential by about  $0.1 \text{ V}$  and increased both oxidation and reduction currents. This behavior can be seen in mediated electrocatalysis.



**Figure 4:** Cyclic voltammograms of bare GC and NB-CH GC electrode with scan rate of  $20 \text{ mV s}^{-1}$  in  $0.5 \text{ M}$  sulfate buffer. Glassy carbon (a) in the absence and (b) the presence of  $0.5 \text{ mM}$  UA; NB-CH GC electrode (c) in the absence and (d) the presence of UA ( $0.5 \text{ mM}$ ).

Differential pulse voltammetry (DPV) was also used to examine the electrochemical behaviors of NB-CH GC electrode. Figure 5 shows the effect of uric acid concentration on the DPV of the modified electrode. According to Figure 5, the height of the anodic peak current increases with uric acid concentration. Figure 5b shows a linear relation between  $I_{pa}$  and UA concentration appears over the range of  $3 \times 10^{-5}$  to  $1.8 \times 10^{-4}$  M, with a linear regression of 0.992. The sensitivity of NB-CH GC electrode was  $0.063 \mu\text{A}/\mu\text{M}$ . The linear dynamic range of GC electrode was  $3.5 \times 10^{-5}$  to  $1.5 \times 10^{-4}$  and the sensitivity of GC electrode was  $0.0074 \mu\text{A}/\mu\text{M}$ . The LODs of NB-CH GC and bare GC electrodes were calculated to be 10 and  $13 \mu\text{M}$ , respectively.

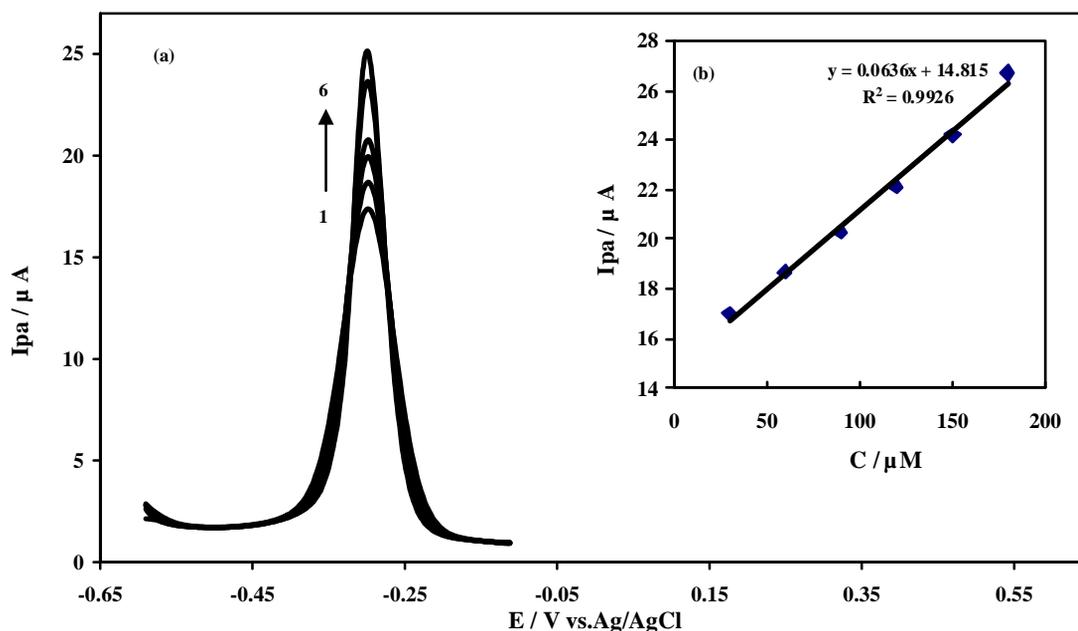
The response time of NB-CH GC electrode was about 10 s and the produced response was stable for a long time. Figure 6 shows the response of electrode for about 600 seconds.

Chronoamperometry along with other electrochemical methods may be used for the investigation of electrode processes at modified electrodes. Figure 7 shows well defined chronoamperograms for the NB-CH GC electrode with a surface coverage of  $2 \times 10^{-8} \text{ mol cm}^{-2}$  in the absence (a) and the presence of (b) 0.5, (c) 1 and (d) uric acid

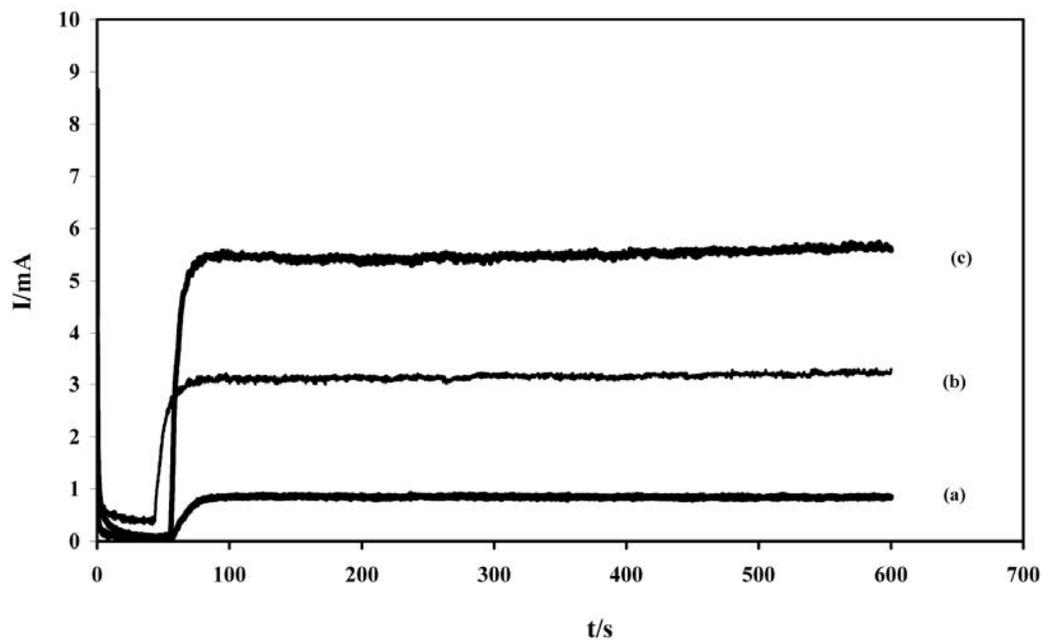
(2 mM) at applied potential steps of 0.35 and -0.1 V vs. Ag/AgCl, respectively. For an electroactive material with a diffusion coefficient of  $D$ , the current corresponding to the electrochemical reaction (under diffusion-controlled condition) is described by Cottrell equation (2) [18]:

$$I = nFAC_0D^{1/2}\pi^{-1/2}t^{-1/2} \quad (2)$$

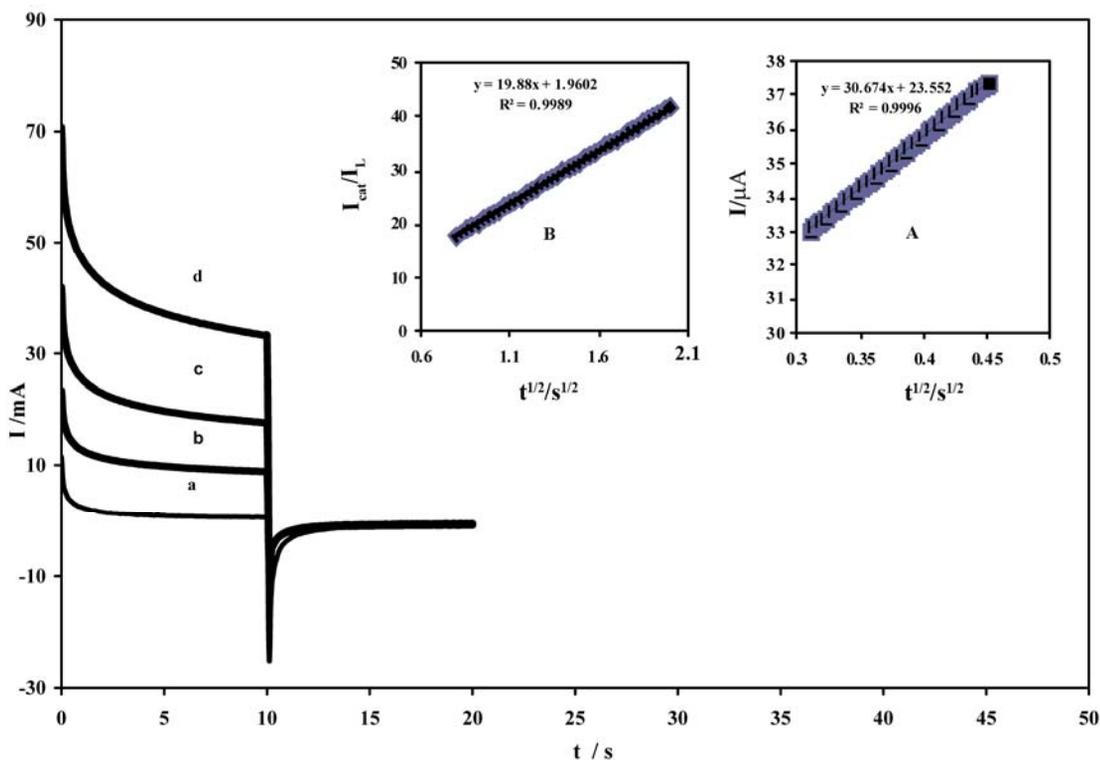
where  $D$  is the apparent diffusion coefficient ( $\text{cm}^2 \text{ s}^{-1}$ ) and  $C_0$  is the apparent bulk concentration ( $\text{mol cm}^{-3}$ ), respectively. Diffusion coefficient is easily calculated from the slope of the plot of  $I$  vs  $t^{-1/2}$ . The mean value of  $D$  was found to be  $9.8 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$  which is in good agreement with the values reported in [19, 20]. At intermediate times ( $t = 2$  to  $4$  s in the current work) in the chronoamperograms, the catalytic current ( $I_{cat}$ ) and the limit current ( $I_L$ ) are determined in the presence and absence of UA respectively; and according to the method described in the literature [21] from the slope of the  $I_{cat} / I_L$  vs  $t^{1/2}$  plot (Figure 7a), we can calculate the value of  $k$  for a given concentration of UA. The value for  $k$  was found to be  $3.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ .



**Figure 5:** a) Differential pulse voltammetry for 1 to 6 (30, 60, 90, 120, 150, 180  $\mu\text{M}$ ) concentrations of UA on NB-CH GC electrode. b) Calibration curve for UA in the range of  $3 \times 10^{-5}$  to  $1.8 \times 10^{-4}$  M in sulfate buffer (0.5 M) as supporting electrolyte (pH=7).



**Figure 6:** Stability of hydrodynamic amperometric response to a) 0.1, b) 0.2, and c) 0.5 mM of UA at NB-CH GC electrode in sulfate buffer (0.5 M).



**Figure 7:** Chronoamperograms of NB-CH GC electrode obtained in the absence (a) and the presence of (b) 0.5 mM, (c) 1.0 mM and (d) 2.0 mM UA in a sulfate buffer (0.5 M) as supporting electrolyte. A) Plot of  $I$  versus  $t^{1/2}$  for UA obtained from chronoamperograms. B) Plot of  $I_{cat}/I_L$  versus  $t^{1/2}$  for UA obtained from chronoamperograms.

### 3.6. Real Sample analysis

In order to evaluate the analytical applications of the new NB-CH GC electrode, some synthetic samples were prepared for the quantification of UA. For this purpose, 10 mL of diluted serum solutions spiked with different concentrations of the standard solutions were prepared and their DPV voltammograms were recorded. According to the standard addition method, uric acid concentration was found to be  $50 \pm 3.5 \text{ mg L}^{-1}$  (for N=3).

### 4. Conclusions

In this paper, we used chitosan as an adsorbent of NB and investigated the electroactivity of NB-CH GC electrode. The electrochemical and electrocatalytic behavior of prepared electrode was studied with different electrochemical techniques. Experimental results showed that this electrode can be used as pH sensor in the pH range of 1-9; also this electrode can be used as a UA sensor. The sensitivity of the prepared electrode was calculated to be  $0.063 \mu\text{A } \mu\text{M}^{-1}$  by DPV technique.

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