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Electrochemical Studies of Sodium Levothyroxine at Surfactant Modified Carbon Paste Electrode

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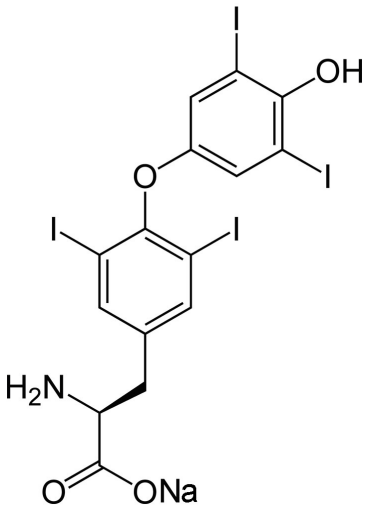
The electrochemical response of sodium levothyroxine at carbon paste electrode in the presence of 0.1M HCl as supporting electrolyte was investigated by the cyclic voltammetry. It showed a well-defined oxidation peak at 0.78V and a sensitive and indiscernible reduction peaks at 0.53V and 0.32V. The effect of concentration and scan rate of sodium levothyroxine was studied. The scan rate effect showed the electrode process is adsorption controlled. The effect of surfactants like Sodium Dodecyl Sulfate (SDS), Cetyltrimethylammonium Bromide (CTAB), and TritonX-100 (TX-100) were studied by mobilizing and immobilizing methods. The concentration effect of all the three surfactants were studied. Among these SDS was showed excellent enhancement in both oxidation peak and reduction peak currents.

Keywords: Sodium levothyroxine, SDS, CTAB, TX100, Carbon paste electrode, Cyclic voltammetry

1. INTRODUCTION

Thyroxine (T4) (3,5,3', 5'-tetraiodothyronine) [Scheme-1] is a derivative of the amino acid tyrosine & is unique in being the only iodine-containing compound of importance [1-5]. Thyroxine is an important biological component produced in the thyroid glands. The practical significances of thyroxine measurements for the diagnosis of hyperthyroidism and hypothyroidism have been known for many years.

The usual methods for the determination of T4 were enzyme immunoassays [6-8], time resolved fluroscence[9], radioimmunoassay(RIA)[10], capillary electrophoresis with laser-induced fluroscence[11], HPLC[12] , chemiluminescence(CL)[13].



Scheme 1. Structure of Sodium Levothyroxine

However these methods have some disadvantages such as expensive instrumentation and time consuming complicated operations. Electrochemical techniques have also been used for the detection of T4. Jacobsen & Fonahn [14] reported a DPP method for the determination of T4 at HMDE. Cathodic reduction of T4 on silver electrode was studied by Iwamoto & Co-workers [15] in comparison with its multi-step reduction at HMDE. Chemically modified carbon paste electrodes are used by Hu's group for the determination of thyroxin in the presence of CTAB [16-18] and also determination of thyroxine at the glassy carbon electrode modified with SWNTs was reported by F.Wang et al [19].

As far as surfactants are concerned, they are a kind of amphiphilic molecules with a polar (hydrophilic) head compatible with water on one side and a long hydrophobic tail compatible with oil on the other side. The applications of surfactants in electroanalytical chemistry have been widely reported [20]. Hu's group [21-24] has introduced surfactants to electroanalytical chemistry to improve the detection limits of some biomolecules. The results showed that the electrochemical responses of these compounds were greatly enhanced in the presence of trace surfactants. They proposed a synergistic adsorption mechanism to interpret these enhancement effects of surfactants.ie, Surfactants might combine with the substrate in certain forms and strengthen their adsorption on the electrode surface, which facilitated the electron or substance transfer between the electrode and the solution and alter the properties of electrode/solution interface and finally influence the electrochemical process of electroactive species [25-28]. Hu and Bard [29] have characterized the adsorption of sodium dodecyl sulfate (SDS) on both charge regulated and hydrophobic substrates by atomic force microscopy measurement. They found that the interaction between SDS and the positively charged electrode surface was a strong function of SDS concentration, Triton X-100 modified carbon paste electrode showed increase in the signal for Dopamine [30]. These results were consistent with the conclusions drawn by Montgometry and Wirth [31] using spectroscopic methods.

As a part of our research work on the electro organic reactions at the surface of the electrodes we extended our work on the modification of carbon paste electrode [32-34,30]. In all the above electrochemical methods [16-19] thyroxine stock solution was prepared in 0.1M NaOH ethanol solution. Where as in this paper unlike the above mentioned, the stock solution was prepared in 2% orthophosphoric acid methanol [pH 4], which makes the solution acidic thereby altering the properties of Thyroxine.

In the present work a simple and sensitive voltammetric method is presented for the detection of T4 based on the increase in the current signal of oxidation and reduction at bare carbon paste electrode in presence three surfactants-viz SDS, CTAB, and Triton-X, similar to the formation of a special ion complex of halogen, I_2Cl^-, SDS, CTAB, Triton-X may form an ion complex with iodine atoms on T4 and thus facilitates both oxidation and reduction. Among these SDS an anionic surfactant showed large peak current compared to CTAB and Triton-X (which showed similar effect) by strongly adsorbing at the surface of a carbon paste electrode via the hydrophobic interaction. The results revealed not only the adsorptive behaviour of SDS but also the influence of preparation solvent of the substrates on the chemical responses at carbon paste electrode in the presence of surfactants.

2. EXPERIMENTAL PART

2.1. Apparatus

Electrochemical measurements were carried out with a model-201 electrochemical analyzer (EA-201 Chemilink system) in a conventional three-electrode system. The working electrode was a carbon paste electrode, having home made cavity of 3mm diameter. A Pt wire and a saturated calomel electrode (SCE) were used as the counter and the reference electrode respectively.

2.2. Reagents

T4 (obtained from Sigma) was dissolved in methanol with 2% of dilute orthophosphoric acid to prepare $5X10^{-4}$ M/L standard stock solutions and stored at 4 degree. SDS was dissolved in water to form $1X10^{-6}$M/L solutions. Other chemicals used were of analytical grade except for spectroscopically pure graphite powder. All solutions were prepared with double distilled water.

2.3. Preparation of carbon paste electrode

The carbon paste electrode was prepared by hand mixing 70% graphite powder and 30% silicon oil by hand mixing in an agate mortar for about 30min to get homogeneous carbon paste. This carbon paste was then packed into the cavity of a Teflon tube electrode (3mm in diameter).

Before measurement the modified electrode was smoothed on a piece of transparent paper to get a uniform, smooth and fresh surface.

3. RESULTS AND DISCUSSION

3.1. The voltammetric behavior of sodium levothyroxine at carbon paste electrode

Fig 1a shows the cyclic voltammogram of T4 at carbon paste electrode, which was investigated in 0.1M HCl. When the potential initially sweeps from 0 to 1.0V a well-defined oxidation peak at 0.78V (O_1) in the positive scan and two reduction peaks at 0.53(R_1) and 0.32V(R_2) on the reversal scan are observed in the first cycle.

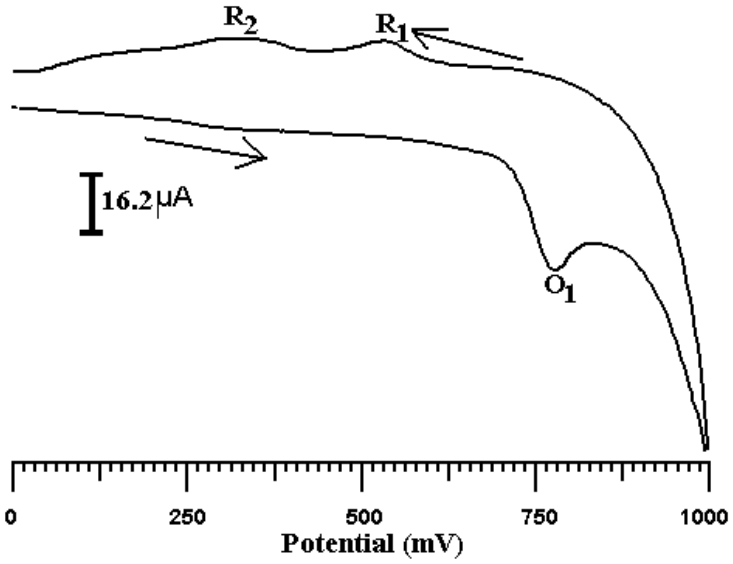


Figure 1a. Cyclic voltammogram of $1X10^{-4}$ M Thyroxine at CPE in 0.1M HCl ; scan rate, 100mV/s.

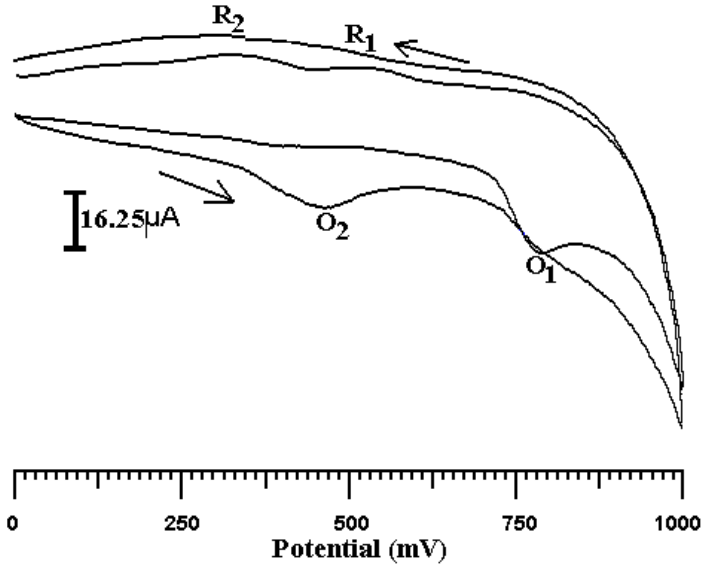


Figure 1b. Appearance of new peak @ around 0.48V after the first scan in the electrochemical response of $1X10^{-4}$ M Thyroxine in 0.1M HCl; scan rate, 100mV/s

In the second and following cycles the peak currents of O_1 and R_1 decrease greatly and a new oxidation peak appears at about 0.4V (O_2). The peak currents of O_1 and R_1 decrease with the increasing of scan number while those of O_2 and R_2 remain stable. These results shows that the electrochemical behaviors of T4 at carbon paste electrode are totally irreversible and that the products are strongly adsorbed on the electrode surface, blocking the mass transfer of T4 from the solution to the electrode surface.

According to Murphy [35], the appearance of O_1 is due to the oxidation of OH on the phenol moiety of T4. From the structure of T4 it is clear that R_1 may be the reduction peak of iodine atoms on T4. The O_2 and R_2 are the electrochemical responses of the product of T4 produced from the oxidation of OH on T4. (Although the proper electrochemical reactions involved in the oxidation/reduction of T4 have been proposed, the hidden relationships between these responses are still unknown). When electrode potential was scanned over the range of 0.5-1.0V, the OH signal (i.e. O_1) is unchanged but no peaks were observed in the reverse scan. Such results prove that reduction of the iodine atoms on T4 is achieved only after the oxidation of OH on T4 because the iodine atoms on the phenol group of T4 are activated after the stable benzene ring is destroyed during the oxidation process.

3.2. Electrochemical response of thyroxine at carbon paste electrode in presence of surfactants

It is well known that surfactants can be adsorbed on solid surfaces to form surfactant film [21,36] which may alter the over voltage of the electrode and influence the rate of electron transfer.

The electrochemical responses of T4 at carbon paste electrode in the presence of trace amount of surfactants onto the surface (immobilized form) as well as into the solution (mobilized form) were studied (Fig.2a to Fig.2f) in 0.1M HCl as supporting electrolyte with 100 mV/s scan rate. The low signal (solid line) is the cyclic voltammogram of T4 in the absence of surfactants. However the voltammetric response is apparently improved (dotted line) in the presence of 10μL of SDS (Fig.2a and Fig.2b), CTAB (Fig.2c and Fig.2d) and TX-100 (Fig.2e and Fig.2f) both in mobilized and immobilized forms respectively. Comparative Cyclic voltammograms of 10μL TX-100, CTAB and SDS are also shown in the Fig.3a(a-d) and Fig.3b(a-d) for both mobilized and immobilized forms respectively When the cationic surfactant CTAB and non-ionic surfactant TX-100 were used, there was increase in both oxidation as well as reduction peak currents both into the solution and onto the surface, shifting the cathodic peak potential to the negative side and anodic peak potential to more positive. But on the contrary when SDS is used large oxidation and reduction peak currents obtained. These results show that anionic surfactants can more effectively promote both oxidation as well as reduction of T4. This may be because T4 is an amphipathic molecule and in strong basic solution carboxyl group in T4 is completely ionized and negatively charged. When cationic surfactant CTAB is added the adsorption of CTAB on the electrode surface may form a positively charged hydrophilic film on the electrode, therefore the oxidation of T4 is facilitated by the cationic surfactant CTAB [17, 15]. This explains that T4 exists in more positively charged by ionization in acidic media i.e. in orthophosphoric acid-methanol T4 exists in cationic form and interacts with negative-charged head groups SDS via electrostatic interactions[37]. Therefore by preparing the Thyroxine in methanol with

2% orthophosphoric acid (pH 4) makes possible the enhancement of both oxidation as well as reduction peaks compared to CTAB and TX-100.

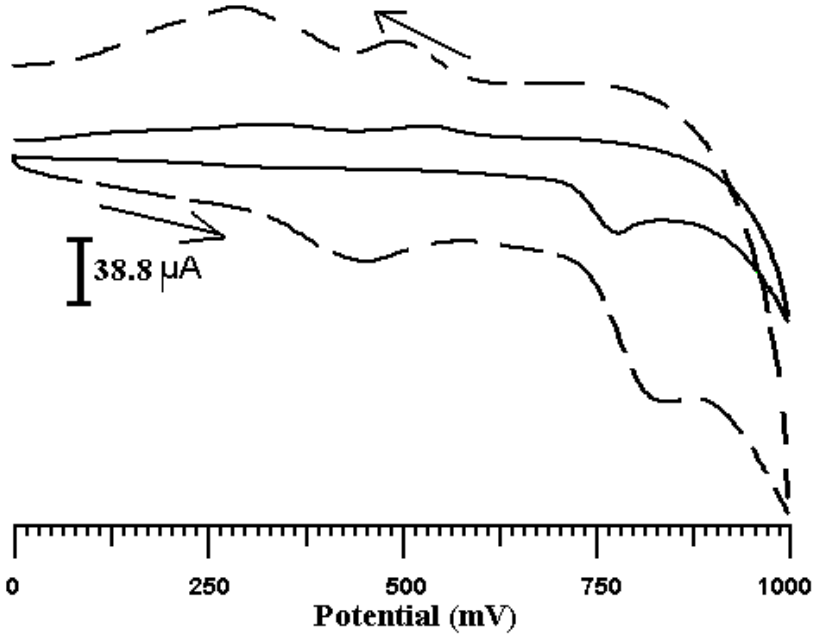


Figure 2a. Cyclic voltammogram $1X10^{-4}$ M Thyroxine at (a) the bare CPE and (b) $1X10^{-5}$M SDS surfactant mobilized CPE.

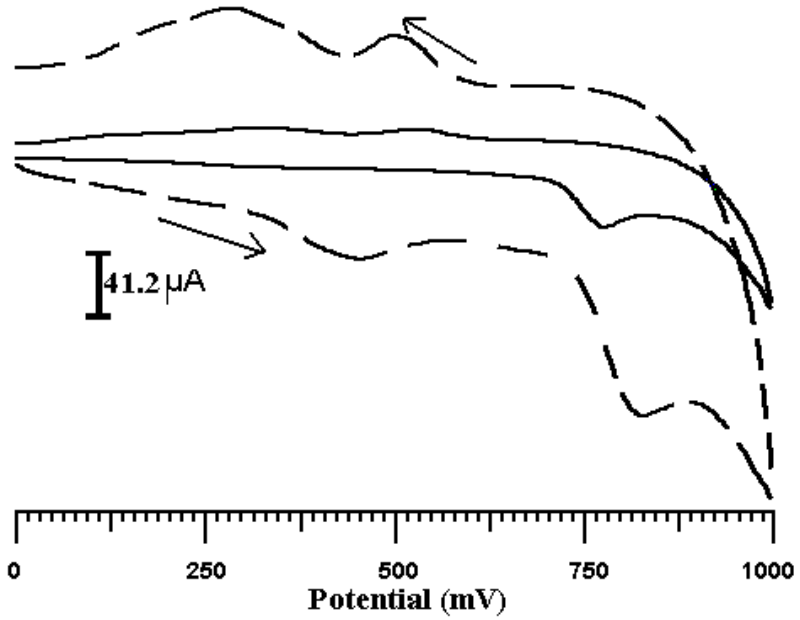


Figure 2b. Cyclic voltammogram $1X10^{-4}$ M Thyroxine at (a) the bare CPE and (b) $1X10^{-5}$M SDS surfactant immobilized CPE.

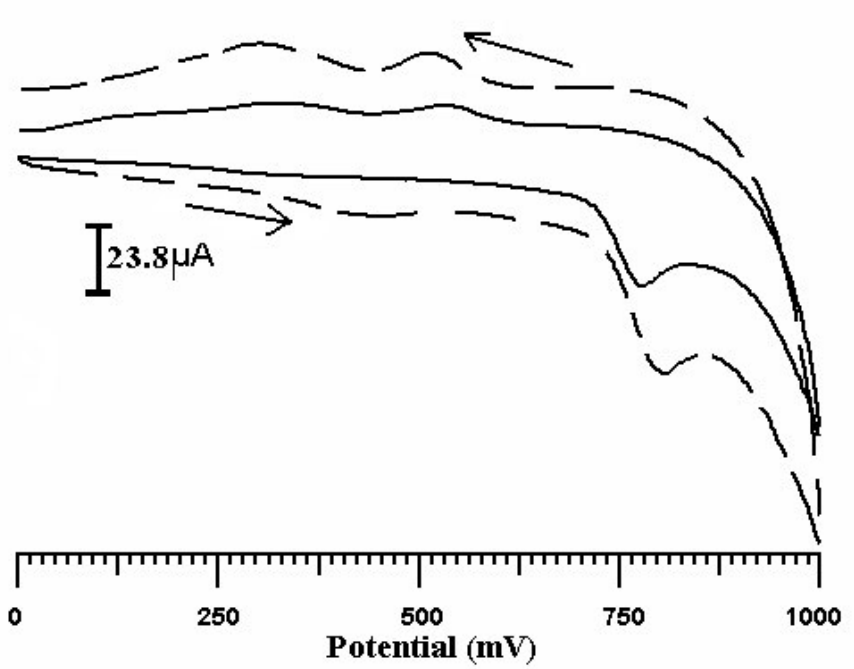


Figure 2c. Cyclic voltammogram $1X10^{-4}$ M Thyroxine at (a) the bare CPE and (b) $1X10^{-5}$M CTAB surfactant mobilized CPE.

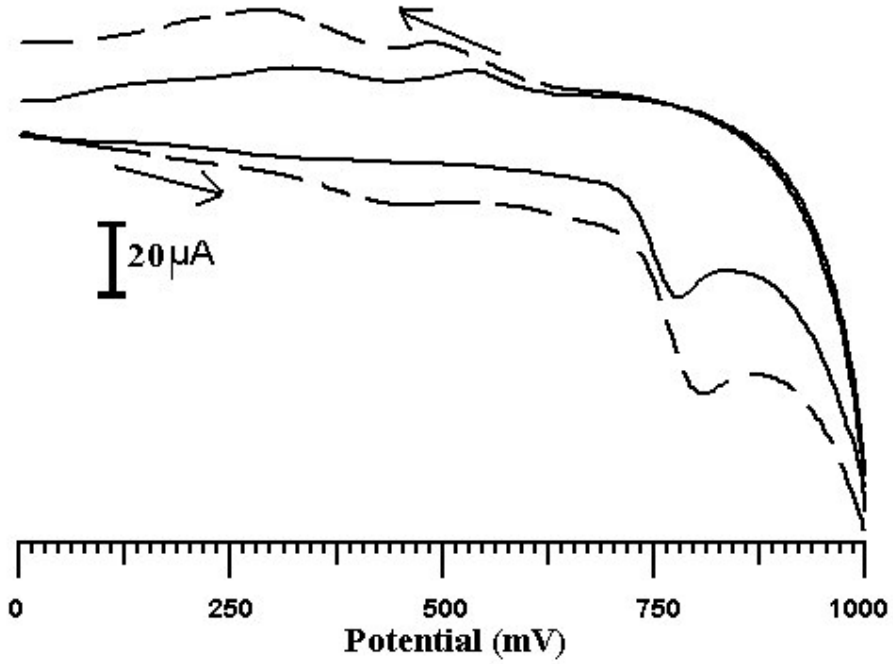


Figure 2d. Cyclic voltammogram $1X10^{-4}$ M Thyroxine at (a) the bare CPE and (b) $1X10^{-5}$M CTAB surfactant immobilized CPE.

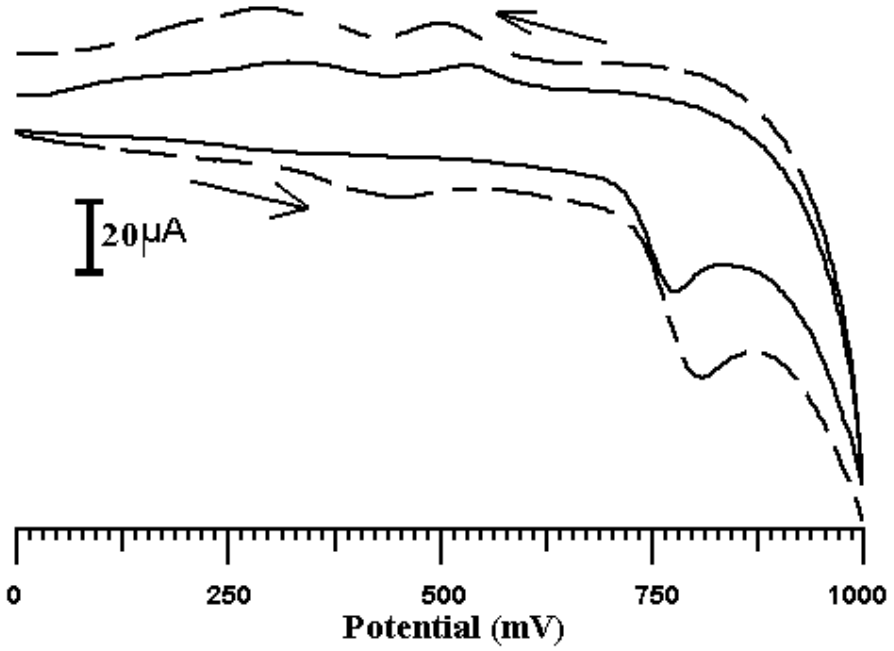


Figure 2e. Cyclic voltammogram $1X10^{-4}$ M Thyroxine at (a) the bare CPE and (b) $1X10^{-5}$M Triton-X surfactant mobilized CPE.

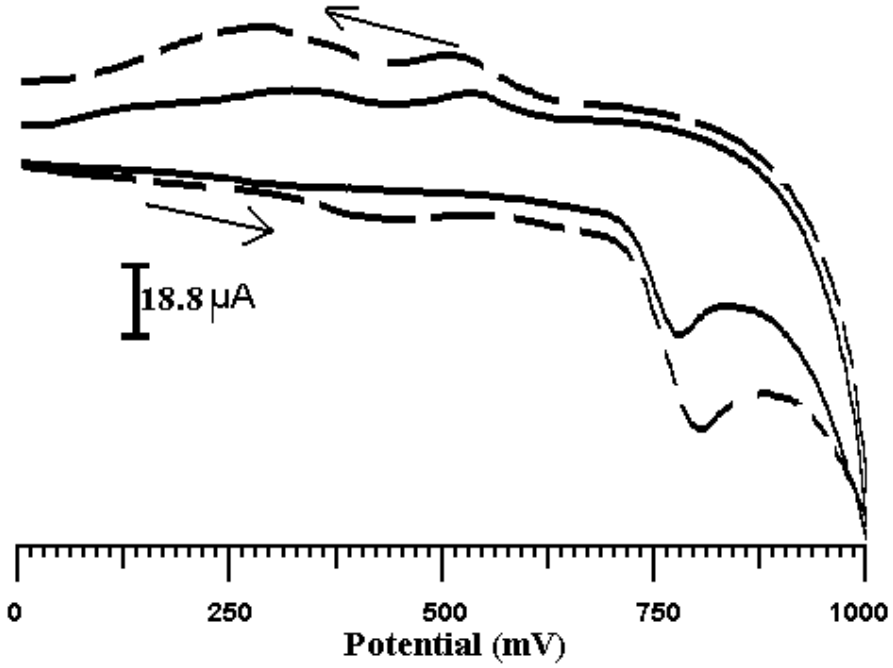


Figure 2f. Cyclic voltammogram $1X10^{-4}$ M Thyroxine at (a) the bare CPE and (b) $1X10^{-5}$M Triton-X surfactant immobilized CPE.

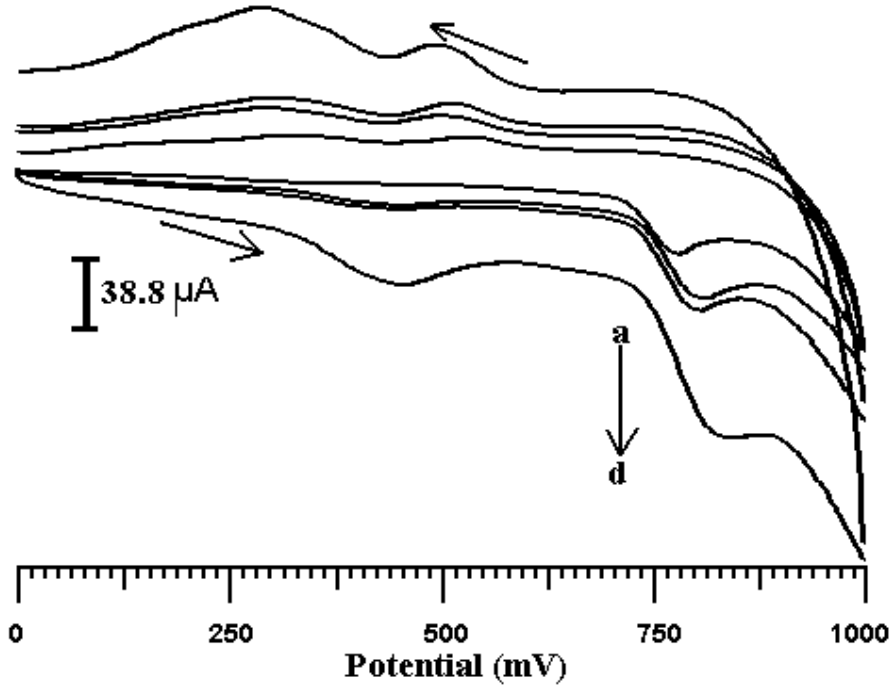


Figure 3a. Cyclic voltammograms for $1X10^{-4}$ M Thyroxine at (a-d). (a) is bare CPE, (b) is $1X10^{-5}$M Triton-X, (c) is $1X10^{-5}$M CTAB and (d) is $1X10^{-5}$M SDS surfactants mobilized CPE.

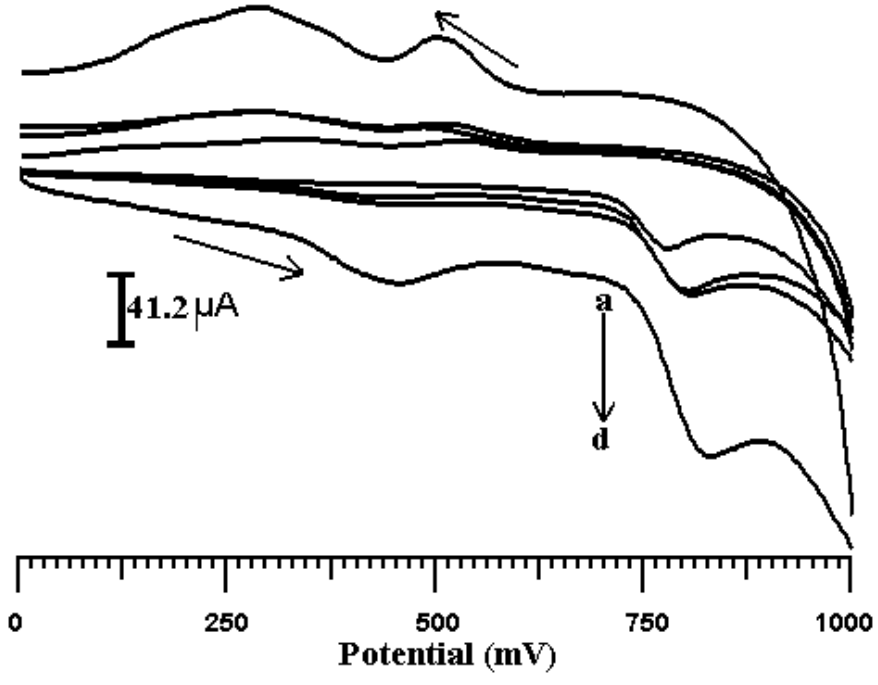


Figure 3b. Cyclic voltammograms for $1X10^{-4}$ M Thyroxine at (a-d). (a) bare CPE and (b) $1X10^{-5}$M Triton-X, (c) $1X10^{-5}$M CTAB and (d)$1X10^{-5}$M SDS surfactants immobilized CPE.

3.3. Effect of surfactant concentration on thyroxine

The effect of surfactant concentration on T4 oxidation/reduction peak currents are shown in fig (Fig.4a and Fig.4b) for both mobilized and immobilized forms. The peak current increases linearly with the concentration of surfactant for all the three. The peak potential of oxidation peak (O_1) shifts towards positive side and the peak potentials (R_1) & (R_2) tends to shift towards negative side in all the three cases. The current response for all the three surfactants in both mobilized and immobilized forms were similar and in both the cases the enhancement in the current response for the SDS surfactant was more compared to CTAB and TX-100.

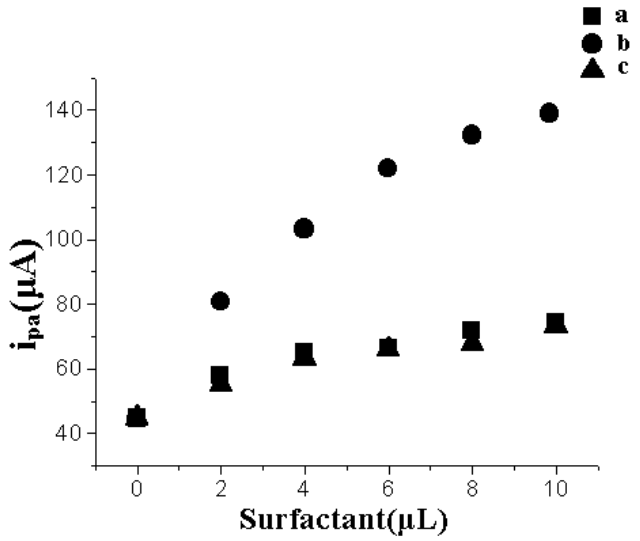


Figure 4a. Effect of surfactant concentration variation on Thyroxine oxidation peak current (a-c); (a) $1X10^{-5}$M Triton-X ,(b) $1X10^{-5}$M CTAB and (c) $1X10^{-5}$M SDS at 0μM, 2μM, 4μM, 6μM, 8μM, 10μM mobilized CPE.

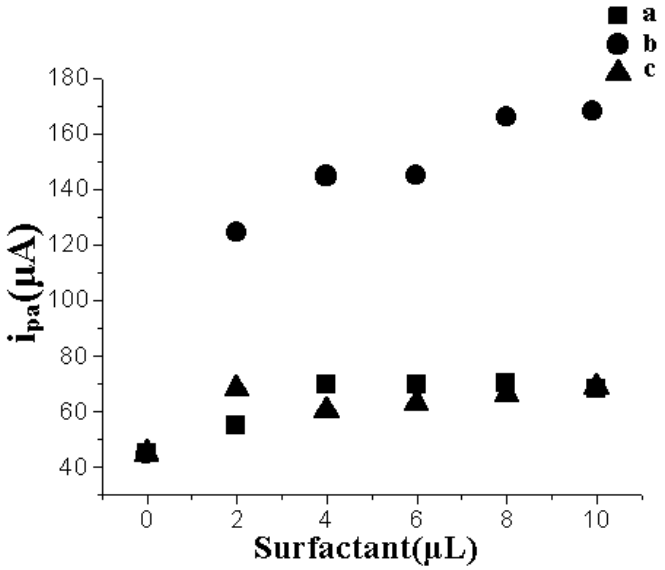


Figure 4b. Effect of surfactant concentration variation on Thyroxine oxidation peak current (a-c); (a) $1X10^{-5}$M Triton-X ,(b) $1X10^{-5}$M CTAB and (d) $1X10^{-5}$M SDS at 0μM, 2μM, 4μM, 6μM, 8μM, 10μM immobilized CPE.

3.4. Effect of concentration of Hydrochloric acid

From the Fig. 5 it is clear that as the concentration of HCl increased from 0.01M to 0.25M the anodic peak potential was decreased from 0.79 V for 0.01M to 0.77 V for 0.1M and again increased from 0.77 V for 0.1M to 0.785 for 0.25M. The oxidation was easier at 0.1M HCl, therefore 0.1M HCl was taken for further studies. The cathodic peak potentials of R_1 peak was negatively shifted and of R_2 positively shifted and the anodic peak potential of O_1 is negatively shifted. Multiple voltammograms indicate that the thyroxine got adsorbed on the surface of the electrode during the redox process. This conclusion is supported by linear nature of i_{pa} vs ν plots [36] (Fig .6a).

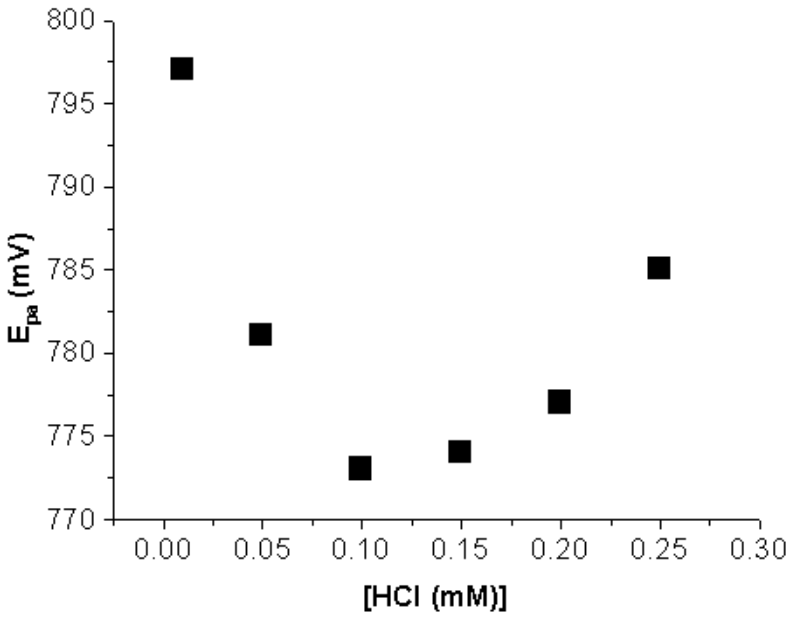


Figure 5. Graph of the different concentration of Hydrochloric acid (a-f ; 0.01M, 0.05 M, 0.1M, 0.15 M, 0.2M and 0.25M).

3.5. Effect of scan rate

The dependence of peak current (i_{pa}) on the scan rate (**v)** was studied in the range of 50-300mV/s, a linear relationship was observed suggesting the adsorption-controlled process of the sodium levothyroxine (Fig.6a). The plot of i_{pa} vs scan rate indicate an increase in peak current with an increase in sweep rate (Fig.6b) confirming that the electrode process at the electrode surface has some adsorption.

3.6.Eeffect of sodium levothyroxine concentration

The cyclic voltammetry showed successive enhancement of peak current on increasing T4 concentration. The plot of peak current vs the respective concentration of T4 was found to be linear in

the range of $2X10^{-4}$ M to $1.2X10^{-3}$ M. The variation of anodic peak current (i_{pa}) with concentration shown in the fig (Fig .7a and Fig.7b)

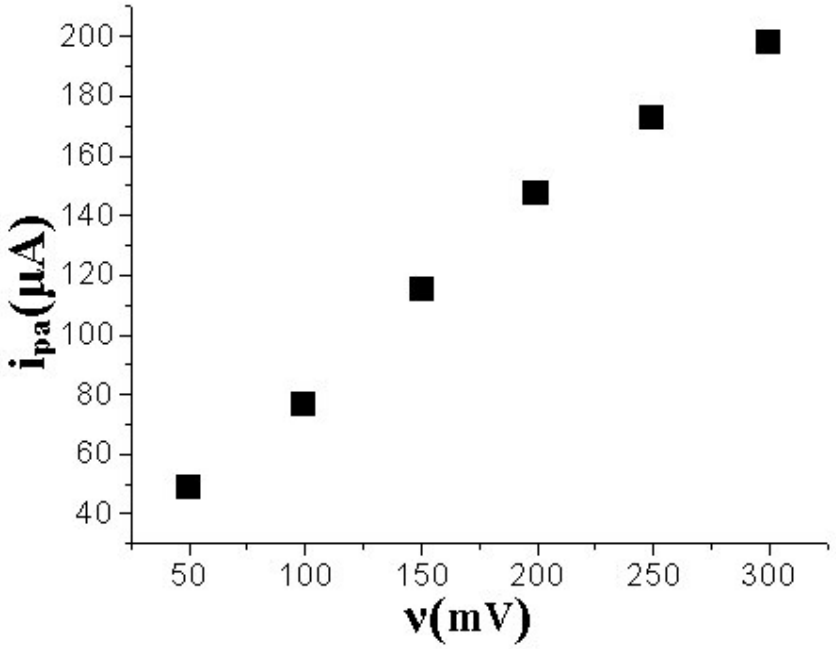


Figure 6a. Cyclic voltammograms of different scan rates (a-f; 50mV/s, 100mV/s, 150mV/s, 200mV/s, 250mV/s and 300mV/s).

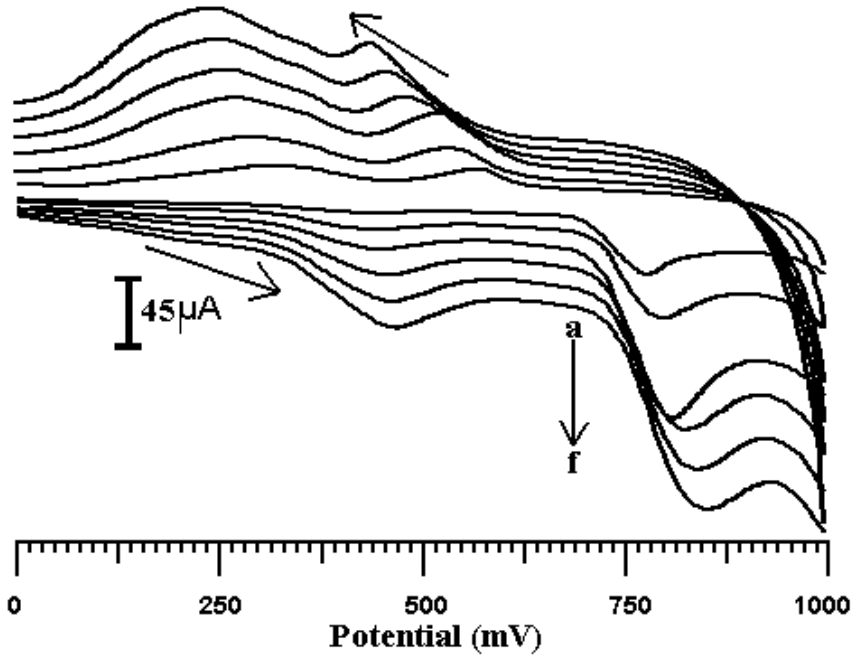


Figure 6b. Graph of current vs scan rate.

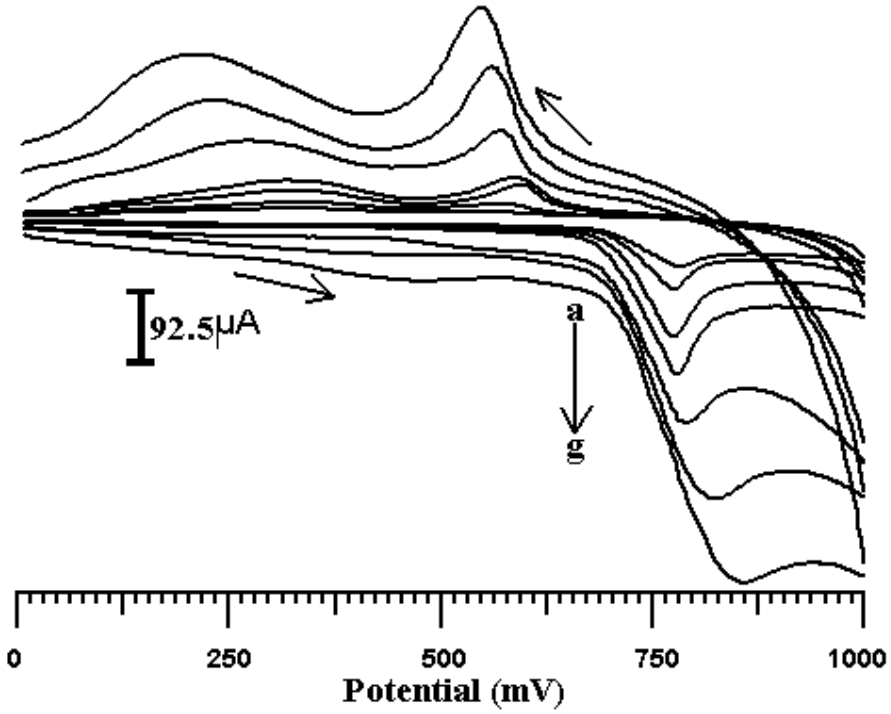


Figure 7a. Cyclic voltammograms for the different concentration of T4 (a-f ; $2X10^{-4}$ M, $4X10^{-4}$ M, $6X10^{-4}$ M, $8X10^{-4}$ M, $1X10^{-3}$M and $1.2X10^{-3}$M).

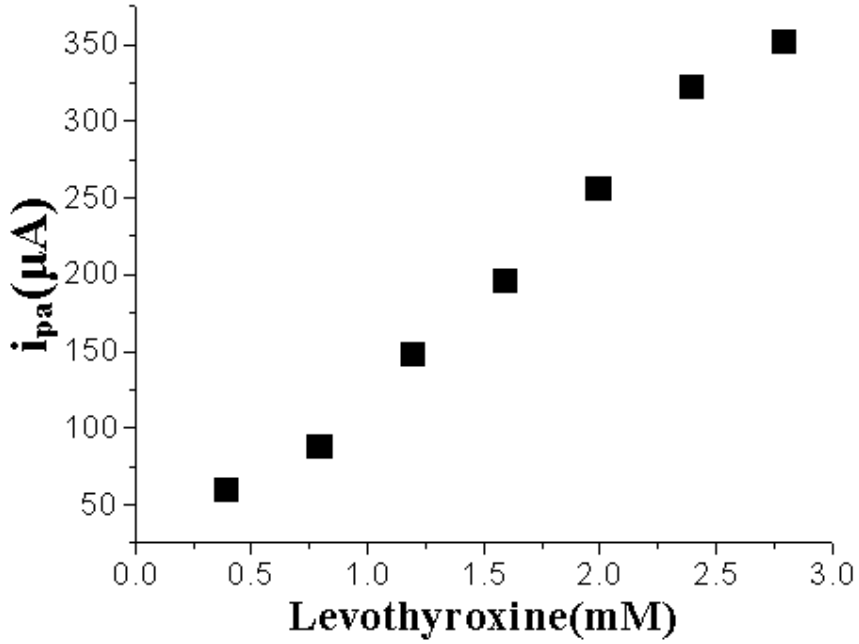


Figure 7b. Graph of different concentration of T4 (a-e ; $2X10^{-4}$ M, $4X10^{-4}$ M, $6X10^{-4}$ M, $8X10^{-4}$ M and $1X10^{-3}$M).

4. CONCLUSIONS

In this work, SDS adsorbs on the CPE surface individually with its hydrophobic C-H chains close to the surface, thereby increasing the surface area and its negative charged head groups directs towards the bulk solution. In acidic media T4 exists in cationic form and interacts with negative charged head groups of SDS through electrostatic interactions. However, the cationic surfactants such as CTAB and non-ionic surfactants such as Triton-X will improve the oxidation/reduction peak current. In conclusion, based on the different existing form of T4 (cationic or anionic) depending on the preparation media, suitable ionic surfactants can be used to improve the sensitivity of determination of T4 using a carbon paste electrode.

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