

# Bentonite (clay montmorillonite) as a template for electrosynthesis of thyroxine

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The results discussed in this paper show that modification of the working electrode surface with clay montmorillonite (bentonite) enhances the iodine redox process and electrosynthesis of thyroxine. This is observed from the well defined quasi-reversible redox peaks obtained in the case of a modified surface as compared to bare carbon. These improvements in redox properties are attributed to the effect of pre-concentration and electrocatalysis. Orientation of clay plates also plays an important role.

**Keywords:** bentonite-modified electrode, iodine redox process, thyroxine electrosynthesis

## 1. Introduction

Clay minerals are heterogeneous materials each having different composition and particle sizes. The use of these materials in developing clay-modified electrodes (CME) emanates from the fact that the materials have well-defined layered structures, flexible adsorption properties and potential as catalysts and/or catalyst supports [1,2]. Moreover, they have higher thermal and chemical stability than the traditional materials that have been used for such modifications including nafion and other polyelectrolytes. Recent investigations towards utilizing CME as photoelectrocatalytic devices, biconductive films, acid catalysis and redox catalysis have been reported [3–7].

CME are electrodes prepared by the deposition of clay films on a conductive substrate to improve the selectivity or the sensitivity of the electrodes towards the solution species by acting as a fast electron transfer media [8]. The electrode modification material of interest in this paper is bentonite which is used as the host matrix. Bentonite is a montmorillonite clay with principal intercalated cations being alkaline earth ions and alkali metal ions and a fixed amount of other components like Al, Fe and SiO<sub>2</sub>. These cations are exchangeable and, because the layer lattice structure is expandable, the ions and large molecules can penetrate between the sheets resulting in increased basal spacing. The ability of large organic molecules to penetrate and orient themselves along the plane of the silicate sheet has been reported [9].

Thyroxine is a derivative of the amino acid tyrosine and is unique in being the only iodine-containing compound of importance. The thyroid gland secretes two significant hormones, thyroxine (90%) and triiodothyroxine (10%), that have a profound effect on the metabolic rate of the body [10–14]. This paper explores a method of synthesizing thyroxine from tyrosine and attempts to determine the catalytic role of bentonite in the above process.

## 2. Experimental

All chemical reagents were used as received without further purification. The acids sulphuric and hydrochloric (SD fine chemicals), amino acids L-thyroxine and L-tyrosine (BDH), the salts ferrous sulphate and potassium ferricyanide (Hopkin and Williams), hydrogen peroxide (Spectra Chemicals) and iodine (Aldrich) were all used as received. The raw bentonite with a mesh size ranging from 150 to 200, cation exchange capacity (CEC) 1.18–1.22 mM/g and a pH of 9.2. The clay montmorillonite was purified as described in [15,16].

The electrochemical instrumentation used in generating the cyclical potential scans comprised a PAR model 173 potentiostat/galvanostat coupled with a logarithmic current converter model 376 that controls the current. This was used in conjunction with a PAR model 175 universal programmer that generated the triangular waves. The output was fed into a PAR RE 0089 X-Y recorder. A three-electrode system was used that consisted of carbon graphite as the working electrode, platinum wire as the auxiliary/counter electrode and saturated calomel electrode (SCE) as the reference electrode.

All the solutions were prepared using triply distilled water. All experiments pertaining to bare carbon graphite electrodes, surface area 0.38 cm<sup>2</sup> were conducted after polishing the latter on a felt polishing cloth containing alumina. The methodology of modifying the electrode surface with bentonite as described in [17] was adopted after polishing the latter as described above. The surface area and thickness of the bentonite-modified electrode were approximately 0.64 cm<sup>2</sup> and 0.16 mm, respectively. The bentonite was ground to increase the surface coverage prior to mixing it with tyrosine.

Tyrosine-loaded bentonite was prepared by dissolving 0.36 g tyrosine in 100 ml distilled water and 20 mM iodine. The solution was then poured into a 200 ml beaker contain-

ing 2.5 g bentonite and the resultant thin slurry stirred and kept in an oven; the temperature was maintained at 95 °C for 48 h. The dry solid residue, coded C1, was ground until a fine powder was obtained and the latter material used to make the modified electrode surface.

### 3. Results and discussion

#### 3.1. Enhancement of iodine redox features on bentonite-modified electrode

The redox chemistry of iodine was monitored by scanning the potential of a graphite working electrode in a solution containing 0.02 M iodine and 1 M sulphuric acid.

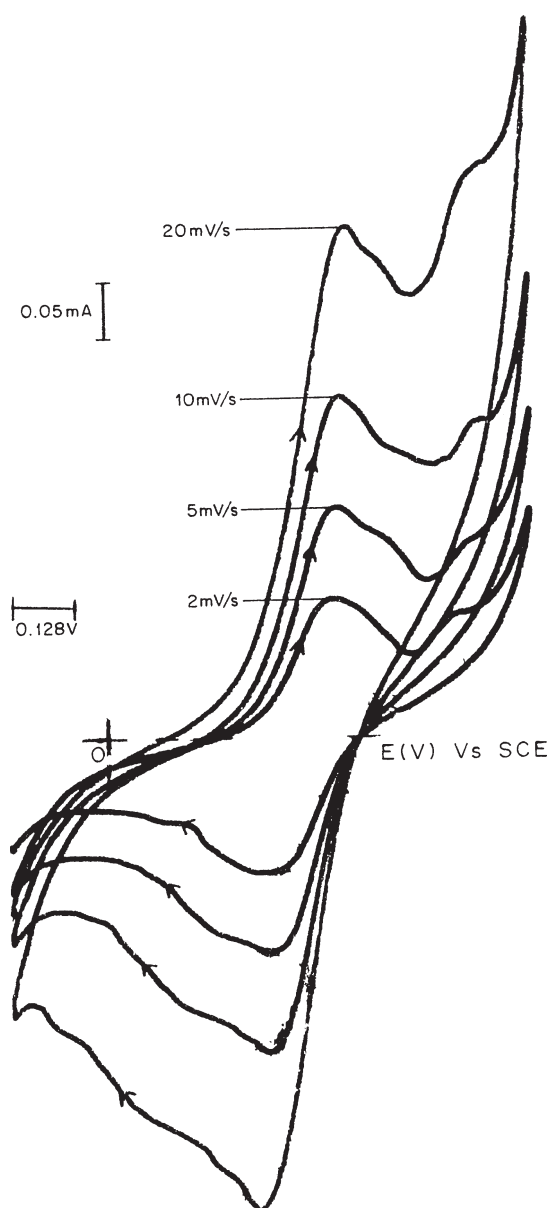


Figure 1. Cyclic voltammetric response for bare carbon electrode obtained in a solution containing 0.02 M iodine and 1 M sulphuric acid. The electrode was transferred to a solution containing 1 M sulphuric acid at different scan rates. The potential limit was from  $-0.2$  to  $0.9$  V.

At this point, it is worth mentioning that not all the iodine was dissolved as its solubility limit was exceeded. The potential was cycled from  $-0.2$  to  $0.9$  V at a scan rate of  $20$  mV/s. The cyclic voltammetric response obtained is shown in figure 1. We observe multiple oxidation peaks and a broad poorly defined reduction peak, consisting of several peaks. The oxidation peaks appear at approximately  $0.39$ ,  $0.46$ , and  $0.60$  V. These peaks are associated with  $I_2$ ,  $I_3^-$  redox processes as they are not observed in the case of bare graphite electrode in  $1$  M sulphuric acid (no iodine).

A plot of the anodic peak current versus scan rate ( $v$ ) yields a linear curve (curve (A), see figure 2) for the peak occurring at  $0.60$  V. The redox process occurring at  $0.46$  V yields a non-linear plot (curve (B), see figure 2). The process at  $0.60$  V is thus due to a surface attached species.

When the working electrode surface was modified using bentonite and then subjected to the same electrolyte solution and potential conditions as the bare graphite electrode case, well defined oxidation reduction peaks occurring at approximately  $0.46$  and  $0.30$  V, respectively, were obtained (see figure 3). This quasi-reversible redox process has a  $\Delta E_p$  of  $160$  mV. A plot of anodic peak current versus square root of scan rate leads to a linear curve, suggesting a diffusion controlled process.

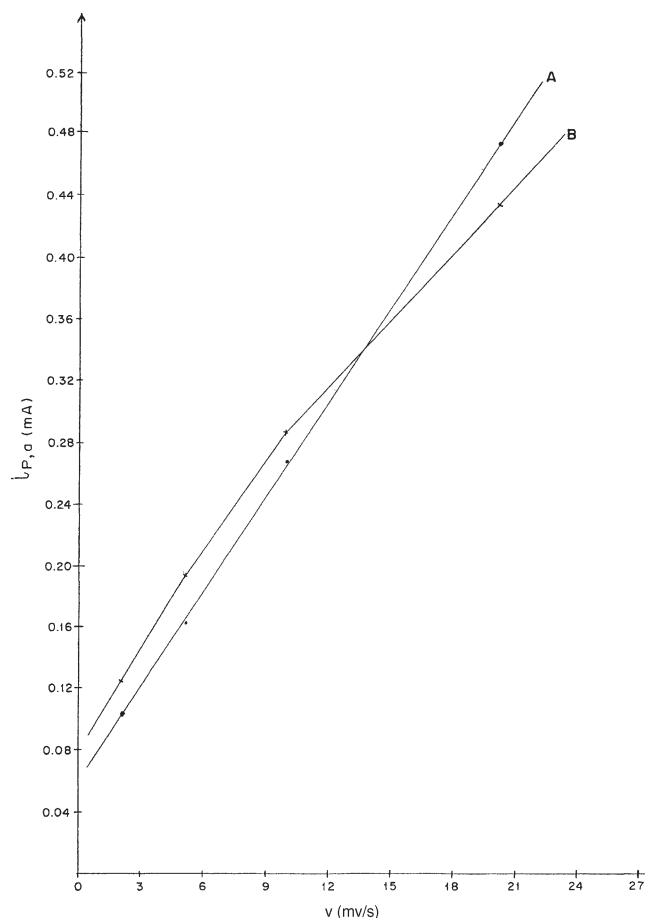


Figure 2. Plot of oxidative peak current,  $i_{pa}$  (mA), versus scan rates,  $v$  (mV): (A)  $0.60$  and (B)  $0.39$  V.

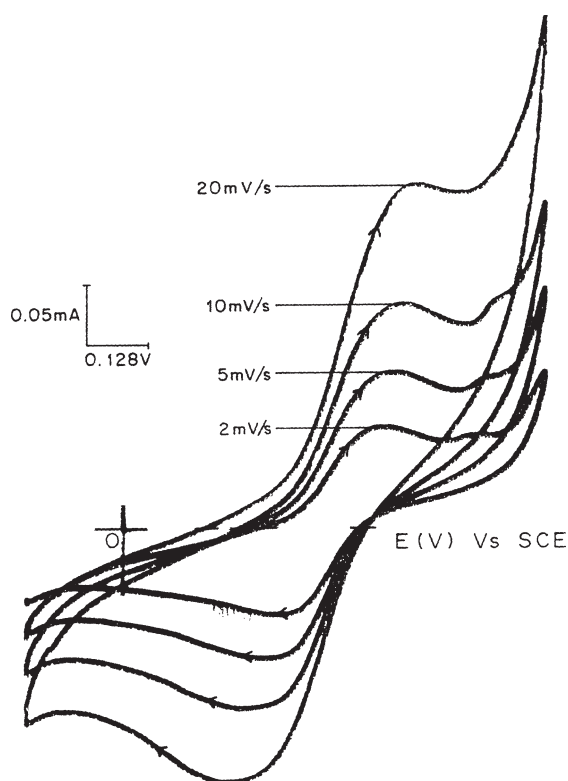


Figure 3. CV for bentonite-modified electrode in 0.02 M iodine and 1 M sulphuric acid, after cycling for 110 min and transferring the electrode to a solution containing 1 M sulphuric acid. Potential limit from  $-0.2$  to  $0.90$  V (at varying scan rates).

The relatively well defined peaks suggest an improvement in electron transfer kinetics probably resulting from electrocatalysis by the electrode modification material. The enhanced redox features can be attributed to several factors. In the first instance the concentration of the electrolyte is higher in the bentonite matrix than in solution, i.e., preconcentration.

Based on the nature of the electrolyte media, the clay montmorillonite has face to face (stacked) rather than edge to face (house of cards) orientation as had been observed previously by other workers from X-ray diffraction measurements [18]. Since  $I^-$  does not interact with the negatively charged clay, it is, therefore, the average size of pores within the film which determines charge transport of the electroactive species. The ratio of the peak currents obtained at the clay-modified and bare electrode would in general be an indicator of the conductivity of the clay film. The enhanced redox features can also be attributed to electrocatalysis by metal ions in the bentonite [16]. With the improved redox features of iodine on bentonite-modified electrode attention was now focussed on the electrosynthesis of thyroxine.

### 3.2. Electrosynthesis of thyroxine on bentonite-modified electrode

The electrosynthesis was commenced by cycling the potential of the bare carbon working electrode in a solution

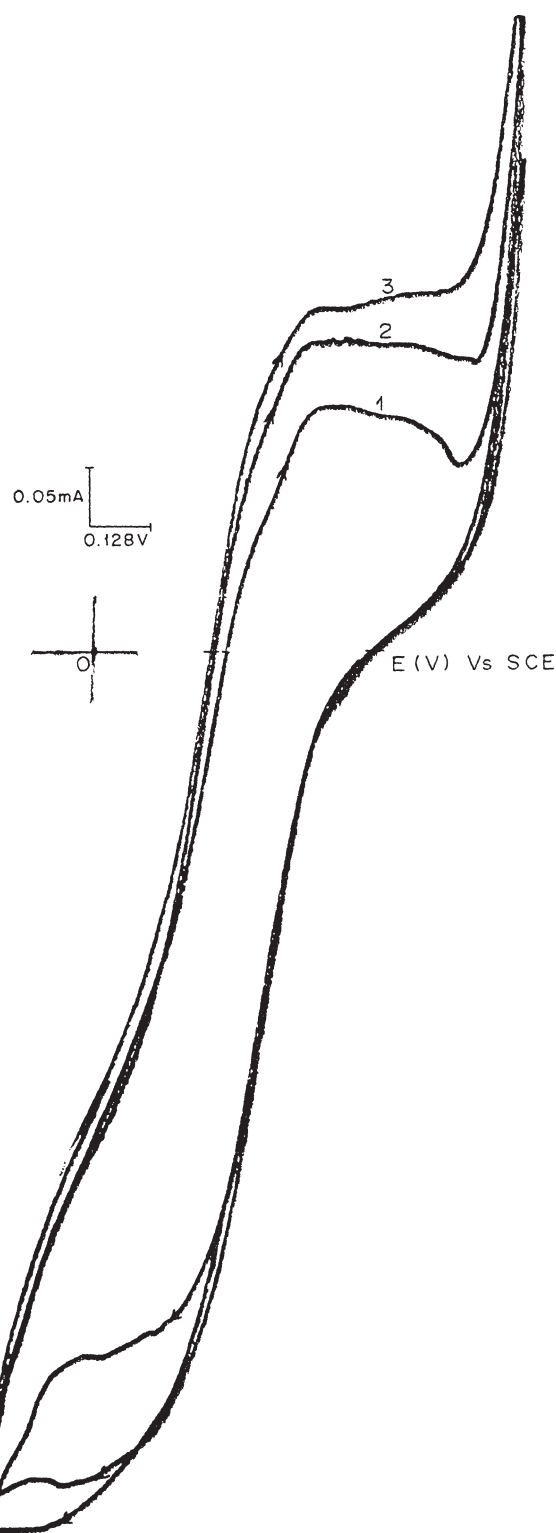


Figure 4. CV for bare carbon graphite electrode in a solution containing 20 mM iodine and 10 mM tyrosine. Potential limit from  $-0.20$  to  $0.9$  V, scan rate  $5$  mV/s. The 2nd and 3rd peaks were recorded after 44.20 and 95.5 min, respectively.

containing 20 mM iodine and 10 mM tyrosine. The potential was cycled from  $-0.2$  to  $0.9$  V at a scan rate of  $5$  mV/s. The resultant cyclic voltammogram (CV) is shown in figure 4. We observe a broad oxidation peak comprising shoul-

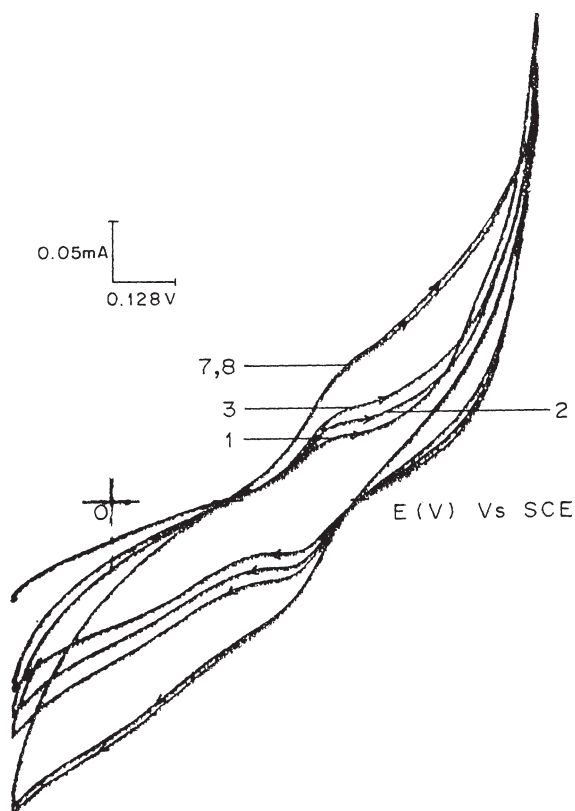


Figure 5. CV response for bare carbon graphite electrode in solution containing 1.25 mM L-thyroxine and 1 M sulphuric acid, potential limit from  $-0.2$  to  $0.90$  V, scan rate  $20$  mV/s.

ders at approximately  $0.44$  and  $0.55$  V; the reduction peak is very poorly defined. The fact that the reduction peak amplitude is considerably higher than that of the oxidation peak suggests a low redox efficiency for the process. This is probably due to the oxidation products diffusing back into the bulk solution. To be able to ascertain whether there was any thyroxine synthesized, we obtained the CV response of L-thyroxine by cycling the potential of the bare carbon working electrode in a solution containing  $1.25$  mM of L-thyroxine in  $1$  M  $H_2SO_4$  from  $-0.2$  to  $0.9$  V at a scan rate of  $20$  mV/s. The resultant CV response is shown in figure 5. We obtain a poorly defined oxidation and reduction peaks at approximately  $0.39$ – $0.47$  and  $0.33$  V, respectively.

Based on the CV data obtained for both cases, it appears likely that we never electrosynthesized sufficient thyroxine in figure 4. The oxidation potential latitude for the broad peak ranges from  $0.4$  to  $0.64$  V. It is worth noting that we had precipitation of iodine on addition of hydrogen peroxide, an oxidant.

Since our main objective was to develop an electrode system which will enhance the redox features of thyroxine obtained from iodine and tyrosine starting materials, it is only after developing such a system that one can go on with electroanalysis to study the various aspects of the hormone, thyroxine. We prepared the bentonite electrode modification material by mixing the latter with tyrosine and iodine, as described in the preparation coded C1 in section 2.

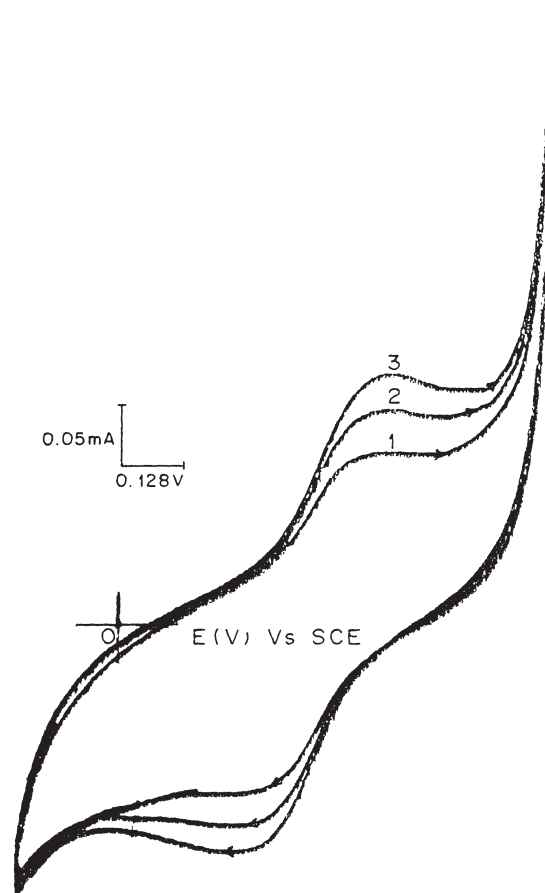
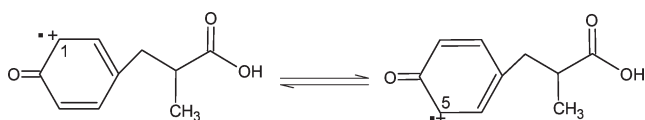
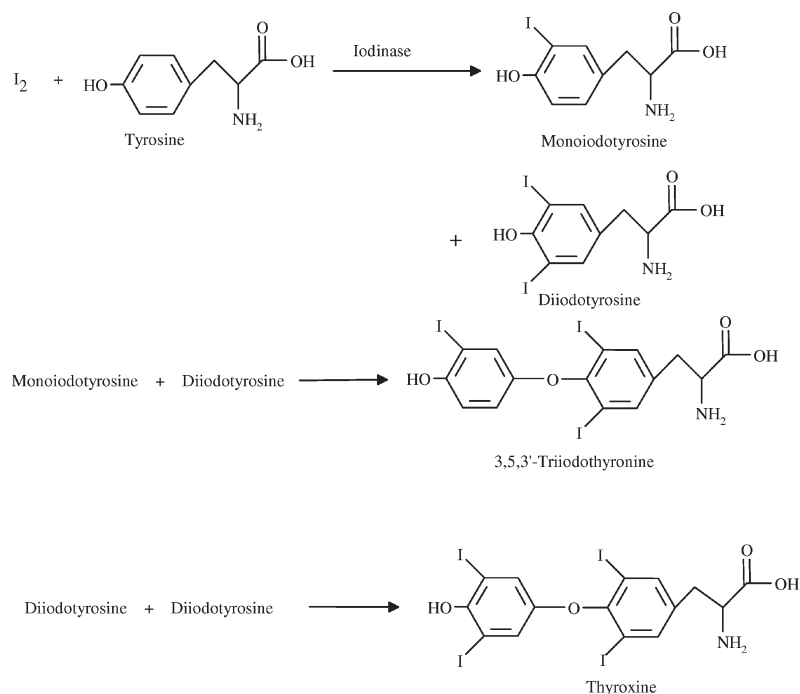


Figure 6. Voltammetric response for C1-modified electrode in  $1$  M sulphuric acid. Potential limit from  $-0.2$  to  $0.90$  V, scan rate  $20$  mV/s.

On cycling the potential of the electrode C1 in  $1$  M  $H_2SO_4$  from  $-0.2$  to  $0.90$  V at  $20$  mV/s, the cyclic voltammetric response obtained is shown in figure 6. We observe now a well defined CV response with the oxidation–reduction potentials occurring at approximately  $0.47$  and  $0.26$  V, respectively. We attribute the redox peaks to thyroxine based on the fact that iodine oxidation/reduction peaks occur at  $0.55/0.192$  V, respectively (see figure 3). The redox potentials for L-thyroxine appear in the region similar to that observed in figure 6, only that they are broad and misshapened. *In situ* IR analysis [19] confirms electrosynthesis of thyroxine. The synthesis of thyroxine is based on the following sequence of actions in the biological systems (see scheme 1). We propose the electrosynthesis to occur via the formation of radical cations in the tyrosine or electrochemical oxidation at the positions, marked 1 and 5, which can then be attacked by the iodine ion (see scheme 2) forming the iodotyrosine derivative, i.e., thyroxine. It was apparent that thyroxine should not display significant dependence on pH. We, therefore, subjected the electrode obtained in figure 6 to solutions of varying pH and monitored the effect on the redox potential. The concentrations of the sulphuric acid solution were  $0.1$ ,  $0.25$ ,  $0.50$  and  $1$  M. We observed no shift in the oxidation potential confirming our earlier assertion (see figure 7).



Since thyroxine is an hormone found in biological systems, we decided to see if iron would have substantial effect on its response as this is an important component of haemoglobin in blood and the possibility of trace amounts of Fe in bentonite being responsible for improved redox properties of thyroxine observed in figure 6.

We, therefore, transferred the electrode used in figure 7 to a solution containing 10 mM ferrous sulphate and 1 M H<sub>2</sub>SO<sub>4</sub>. We cycled the potential from -0.2 to 0.9 V at 20 mV/s. We observed an enhancement of the oxidation and reduction peak currents (see figure 8). The oxidation and reduction peak potentials remained unchanged. The enhanced peak current is probably due to the fact that at very low electrolyte concentration, the orientation of the clay plates is face to face as opposed to face to edge, thus allowing easy mobility for the redox species with the bentonite matrix.

In a separate analysis attempts were made to monitor the synthesis of thyroxine systems from a media containing iodine and tyrosine over a period over 2880 h by obtaining the CV from the electrolyte media on a bare carbon graphite electrode at specific time. Though new peaks kept surfacing and disappearing during the analysis period, the redox peak obtained did not match those obtained from L-thyroxine analysis. We, therefore, concluded that thyroxine had not been synthesized. The CV response obtained after 2880 h is shown in figure 9.

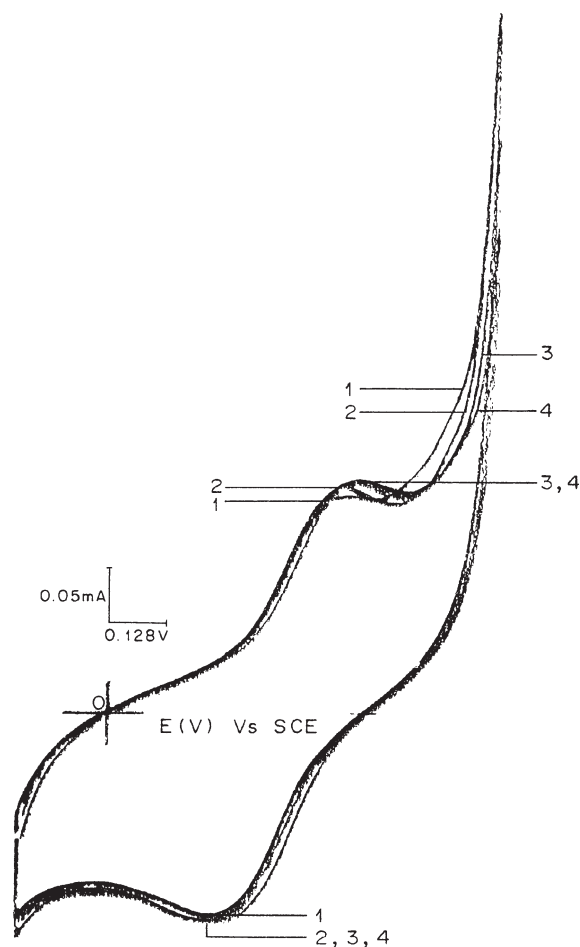


Figure 7. pH dependence studies of C1-modified electrode in varying concentrations of sulphuric acid (1, 2, 3, and 4 representing 0.1, 0.25, 0.50 and 1 M H<sub>2</sub>SO<sub>4</sub>, respectively).

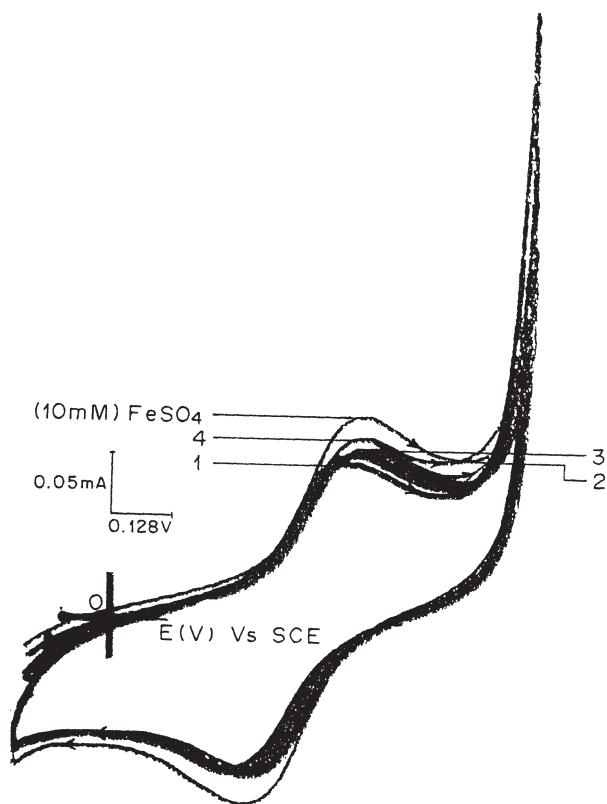


Figure 8. Cyclic voltammetric response obtained on transferring the electrode in figure 7 to a solution containing 10 mM ferrous sulphate and 1 M sulphuric acid. Potential limits from  $-0.2$  to  $0.90$  V, scan rate  $20$  mV/s.

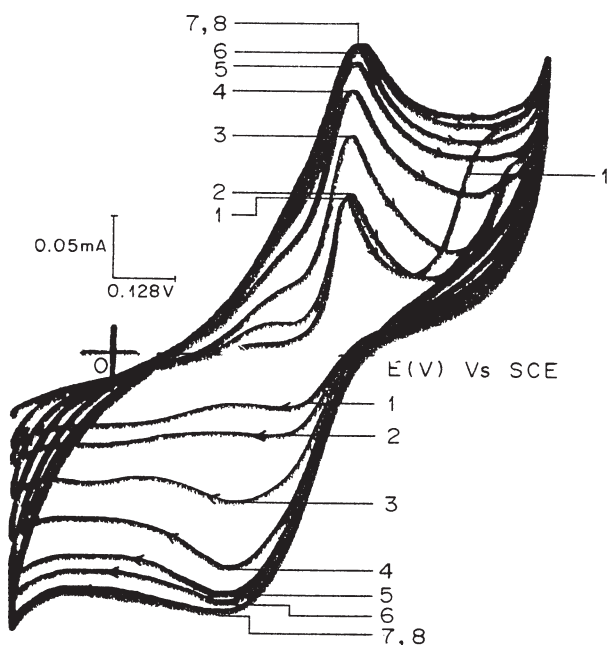


Figure 9. CV response obtained after 2880 h of bare carbon graphite electrode in an electrolyte media containing 10 mM tyrosine, 20 mM iodine and 1 M sulphuric acid (peaks observed over a period of 165 min).

#### 4. Conclusion

The modification of the working electrode surface with bentonite greatly improves the redox process of iodine as a result of preconcentration and electrocatalysis. We also observed that thyroxine can be electrosynthesized on the bentonite-modified electrode, unlike in the case of a bare carbon electrode whereby, after over 2880 h, thyroxine response was not observed. Work is currently in progress to establish the effect of pillared-clay-modified electrode on the electrosynthesis of thyroxine.

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