Enhanced Reduction and Determination of Trace Thyroxine at Carbon Paste Electrode in the Presence of Trace Cetyltrimethylammonium Bromide

Chengguo HU,* Qiong HE,** Qing LI,* and Shengshui HU*†

*Department of Chemistry, Wuhan University, Wuhan 430072, China

**Department of Chemistry, Qujing Normal College, Qujing 655000, China

The electrochemical response of thyroxine (T_4) at a carbon paste electrode (CPE) in the presence of cetyltrimethylammonium bromide (CTAB) was investigated. It gave a well-defined oxidation peak at 0.80 V and a sensitive reduction peak at 0.40 V. Compared with the indiscernible signal in the absence of CTAB, the reduction current of T_4 at CPE was greatly enhanced in the presence of CTAB, due to the interactions between T_4 , CTAB and the hydrophobic electrode surface. The electrode process of T_4 was explored by cyclic voltammetry and chronocoulometry. The effect of surfactants on the reduction reaction proved that bromide ions (Br^-) in CTAB might form special ion complexes with T_4 via a special interaction with the iodine atoms on T_4 , which would activate the reduction of T_4 . The sensitive and selective reduction of T_4 in this system was applied to the determination of T_4 in drugs; a detection limit of 6.5×10^{-9} M was obtained ($\sigma = 3$).

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Introduction

Thyroxine (T_4) is an important substance produced in thyroid glands. The usual methods for the determination of T_4 were immunoassays.¹⁻⁶ However, these methods have some disadvantages, such as expensive instrumentation and complicated operations. The detection of T_4 has also been achieved by electrochemical techniques. T_4 was determined by differential pulse polarography at a hanging mercury drop electrode (HMDE).⁷ The cathodic reduction of T_4 on silver electrode was studied by Iwamoto and co-workers.⁸ In comparison with its multi-step reduction at HMDE, T_4 underwent a one-step reduction on silver; eight electrons were involved in this reduction. T_4 in urine has been determined by cathodic stripping square-wave voltammetry at HMDE; a low detection limit of 2.71 ng/ml was achieved.⁹ However, the employment of a mercury electrode is unsuitable in the interests of environment protection. The successful use of noble metals as electrode materials to catalyze the reduction of T_4 may arise from the special interactions of halogen with these metals. No works on the determination of T_4 at solid electrodes have been reported, except for silver electrodes.⁸ This may be attributed to poor electrochemical responses of T_4 at these kinds of electrodes.

Surfactants are a kind of amphiphilic molecules with a polar head on the one side and a long hydrophobic tail on the other. The applications of surfactants in electrochemistry and electroanalytical chemistry have been widely reported.¹⁰ Hu's group¹¹⁻¹⁴ has used surfactants in electroanalytical chemistry to improve the detection limits of some biomolecules. They

proposed a "synergistic adsorption" mechanism to interpret the enhancement effect of surfactants. Digua *et al.*¹⁵⁻¹⁷ mixed the amphiphile hexadecyl sulfonic acid into carbon paste to produce a surfactant-modified carbon paste electrode. This electrode exhibited strong cation exchange property and an improved electron transfer rate between the substrates and the electrode. Falaras *et al.*¹⁸⁻²⁰ prepared an organoclay-modified glassy carbon electrode coated by a cationic surfactant bilayer. They found that this electrode possessed anion-exchange ability and could accumulate both negatively charged metal complexes and neutral redox active reagents. We²¹ have developed a cetyltrimethylammonium bromide (CTAB)-modified carbon paste electrode based on the surface modification methods. The electrochemical response of $K_4Fe(CN)_6$ at this electrode revealed that CTAB formed a stable monolayer on the electrode surface based on the hydrophobic interaction between the hydrophobic long chain of CTAB and the paraffin oil in carbon paste.

In this paper, a simple and sensitive voltammetric method is presented for the detection of T_4 based on the enhanced reduction of T_4 at CPE in the presence of CTAB. Similar to the formation of a special ion complex of halogen, I_2Cl^- , Br^- in CTAB may form an ion complex with iodine atoms on T_4 and thus facilitate its reduction. This assumption was confirmed by the various electrochemical responses of T_4 at CPE in the presence of surfactants. As a method for the detection of T_4 , the reduction process was more suitable than the oxidation process because the selectivity of the reduction was much higher.

Experimental

Apparatus

Electrochemical measurements were carried out with a CHI

† To whom correspondence should be addressed.
E-mail: sshu@whu.edu.cn

Fig. 1 Electrochemical responses of 5×10^{-6} M thyroxine at CPE in 0.1 M HCl: A, in the presence of 6×10^{-5} M CTAB; B, in the absence of 6×10^{-5} M CTAB. Potential scan direction: from 0.0 to 1.0 V; scan rate, 100 mV/s; accumulation, 5 min at an open circuit.

Fig. 2 Schematic representation of T_4 structure.

830 electrochemical analyzer (Shanghai Chenhua Co., China) in a conventional three-electrode system. The working electrode was a carbon paste electrode. A Pt wire and a saturated calomel electrode (SCE) were used as the counter and the reference electrode, respectively.

Reagents

T_4 stock solutions (5×10^{-4} M) were prepared by adding T_4 (obtained from Sigma, USA) to 0.1 M NaOH ethanol solution; they were kept in darkness at 4°C . Surfactants (purchased from Shanghai Reagent Co., China) were dissolved in water to form 1×10^{-2} M solutions. Other chemicals used were of analytical grade, except for the spectroscopically pure graphite powder. All solutions were prepared with doubly distilled water and all chemicals were used without further purification. Thyroid gland tablets were the gift of Ji'nan Biochemical Pharmaceutical Co., China.

Preparation of carbon paste electrode

A 25- μL volume of paraffin oil was added to 100 mg of spectroscopically pure graphite powder to prepare a homogeneous carbon paste. This carbon paste was then packed into a piston Teflon-tube electrode (4.5 mm in diameter). Prior to measurement, the modified electrode was smoothed on a piece of transparent paper to get a fresh surface.

Procedure

Measured volumes of 0.1 M HCl and 60 μL CTAB solution were pipetted into an electrochemical cell. Then the standard solution of T_4 was added to the cell to form a 10 ml mixture solution. An accumulation potential of 0.9 V was applied to the working electrode for 5 min while the solution was stirred. The voltammograms of T_4 in the potential range of 0.0–1.0 V were recorded. Four apparent peaks appeared, of which the sensitive reduction peak at about 0.40 V was used for the determination

Fig. 3 Multicyclic voltammograms of 5×10^{-6} M T_4 in 0.1 M HCl at CPE in the presence of 6×10^{-5} M CTAB. Potential scan direction: from 1.0 to 0.0 V; scan rate, 100 mV/s; accumulation, 5 min at an open circuit.

of T_4 . The cell was kept under darkness during all measurements to avoid the decomposition of T_4 .⁸

Results and Discussion

Cyclic voltammograms of T_4 in acidic media at CPE in the presence of CTAB

Figure 1A shows the cyclic voltammograms of T_4 at CPE, which were investigated in 0.1 M HCl containing 6×10^{-5} M CTAB. When the potential initially sweeps from 0 to 1.0 V, a well-defined oxidation peak at 0.80 V (O_1) in the positive scan and two reduction peaks at 0.40 V (R_1) and 0.27 V (R_2) on the scan reversal are observed in the first cycle. In the following cycles, the peak currents of O_1 and R_1 decrease greatly and a new oxidation peak appears at about 0.48 V (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 indicate that the electrochemical behaviors of T_4 at CPE are totally irreversible and that the products are strongly adsorbed on the electrode surface, blocking the mass transfer of T_4 from the solution to the electrode surface. According to Murphy,²² the appearance of O_1 is due to the oxidation of OH on the phenol moiety of T_4 . From the structure of T_4 (Fig. 2), it is clear that R_1 may be the reduction peak of iodine atoms on T_4 , since only iodine atoms could be electrochemically reduced in this potential window. As for O_2 and R_2 , they are ascribed to the electrochemical responses of the product of T_4 , *i.e.*, the hydroquinone-benzoquinone redox system produced from the oxidation of OH on T_4 .²² In the absence of CTAB, the signal of T_4 at bare CPE hardly changes except for the greatly depressed R_1 and the slight shift of oxidation potential of O_1 to the positive direction (Fig. 1B). These results indicate that CTAB can greatly enhance the reduction current of T_4 and produces negligible effect on the oxidation reaction.

Mechanism for the reduction/oxidation of T_4 at CPE in the presence of CTAB

Although the proper electrochemical reactions involved in the reduction/oxidation of T_4 have been proposed, the hidden relationships between these responses were still unknown. Figure 3 shows the cyclic voltammograms of T_4 at CPE in the presence of 6×10^{-5} M CTAB when the potential initially sweeps from 1.0 to 0.0 V. The peak current and the peak

Fig. 4 Effect of initial potential (IP) on the voltammograms of T₄: a, 600 mV; b, 680 mV; c, 700 mV; d, 730 mV; e, 850 mV; f, 900 mV; g, 1000 mV. Other conditions are the same as in Fig. 3.

Fig. 5 Variations of peak currents with initial potential (IP) for O₁ (○) and R₁ (●). Other conditions are the same as in Fig. 3.

potential of R₁ have almost the same values as those in Fig. 1A. However, in the reverse scan, the peak current of O₁ is significantly repressed and tends to remain stable in the following cycles, close to the value of the second cycle in Fig. 1A. These results can be interpreted as the strong adsorption of the reduction product of T₄ at the electrode surface. When the electrode potential was scanned over the range of 0.6 – 1.0 V, the signal of OH changed little, while no apparent peaks were observed in the range of 0.6 – 0.0 V (not shown). Such results suggested an inevitable relationship between the oxidation of OH and the reduction of the iodine atoms on T₄. Figure 4 shows the variations of the voltammetric responses of T₄ with the initial potential (IP). It is obvious that the initial potential has remarkable effects on the reduction and the oxidation peak currents of T₄, while it shows negligible effects on the peak potentials. The effects of initial potential on reduction/oxidation peak currents are represented in Fig. 5. When the initial potential is more negative than 0.6 V, R₁ is undiscerned and O₁ is large. However, when the initial potential arrives at 0.68 V, where the oxidation of OH begins (see O₁ in Fig. 4), the reduction peak of R₁ appears. With the further shift of the initial potential to the positive direction, the peak current of R₁ increases correspondingly except for a small drop of peak current at initial potentials more positive than 0.9 V. The variations in the oxidation of OH with the positive shift of initial potential are different. The peak current of O₁ firstly increases with the positive shift of initial potential up to 0.68 V. When the initial potential approaches 0.68 V, the peak current

Fig. 6 Chronocoulometry at CPE in the presence 6×10^{-5} M CTAB for: (a) the oxidation process of 5×10^{-6} M T₄, initial potential 0.6 V, final potential 0.9 V, pulse width 4 s; (b) the reduction process of T₄, initial potential 0.5 V, final potential 0.35 V, pulse width 0.5 s. The solid line curves stand for the presence of 5×10^{-6} M T₄ while those in dot lines are obtained from the blank solutions. Insets show the linear relationships between charges (Q) and the square roots of time ($t^{1/2}$) (background subtracted).

of O₁ arrives at a maximum value. The further increase of initial potential leads to a decrease of the peak current. It is obvious that the oxidation of OH is apparently restrained when the reduction of T₄ begins. These results prove that the reduction of the iodine atoms on T₄ is achieved only after the oxidation of OH on T₄. The above conclusion is authentic, because the iodine atoms on the phenol group of T₄ are activated after the stable benzene ring is destroyed during the oxidation process and are reduced through the inducement effect of Br⁻ in CTAB.

Chronocoulometry was also employed to characterize the electrochemical responses of T₄ at CPE in the presence of CTAB. Figure 6 shows the chronocoulometry of the oxidation of OH (Fig. 6a) and the reduction of iodine atoms (Fig. 6b) on T₄. It is clear that the charges (Q) have linear relationships with the square roots of time ($t^{1/2}$) for both the oxidation and the reduction reactions (background subtracted; insets in Figs. 6a and 6b). According to the integrated Cottrell equation:

$$Q = \frac{2nFAC^bD^{1/2}t^{1/2}}{\pi^{1/2}} \quad (1)$$

where n is the number of the electrons transferred in the studied electrochemical reaction, A is the working electrode area, C^b is the bulk solution of the substrate, D is the diffusion coefficient of the substrate, and the other symbols have their usual significance. The value of n can be obtained when the other parameters in Eq. (1) are known. Because the reduction and the oxidation of T₄ show negligible effects on the dimension of T₄,

Fig. 7 Voltammograms obtained at glassy carbon electrode (a, b) and at carbon paste electrode (c, d) in 0.1 M HCl in the presence of 6×10^{-5} M CTAB for blank solutions (a, c) and for 5×10^{-6} M T_4 solutions (b, d). Other conditions are the same as in Fig. 1.

the diffusion coefficients in the oxidation and the reduction processes of T_4 can be assumed to possess the same value. Then, under the same experimental conditions, the number of electron transfers for the reduction of T_4 can be deduced if n of OH oxidation can be obtained. Under this condition, the ratio of electron transfer between the reduction and the oxidation of T_4 deduced from Fig. 5 and Eq. (1) is 2.078. On the assumption that two electrons were transferred in the oxidation of OH on phenol,²² the electron transfer in the reduction of T_4 was four. Because the effect of the OH oxidation of T_4 on the phenyl ring in the middle position can be neglected, it is rational to consider that the reduction only occurs on the phenol ring, *i.e.*, two iodine atoms are involved in the reduction of T_4 and reduced to iodide ions.

Effects of CTAB on the reduction of T_4

Compared with the low reduction current in the absence of CTAB, the reduction current of R_1 at CPE in the presence of CTAB was greatly enlarged. However, this enhancement effect of T_4 reduction was not applicable to glassy carbon electrode (GCE). Figure 7 shows the cyclic voltammograms of T_4 at different electrodes. T_4 exhibits a poor electrochemical response at GCE in the presence of CTAB. Although the reduction and the oxidation of T_4 are observed, the peak currents are low and the background is large and anomalous. On the contrary, the current response of T_4 at CPE in the presence of CTAB is well-defined and the background is greatly restrained. From the above discussions, one can conclude that the enhanced reduction of T_4 is accomplished through the interactions between T_4 , CTAB and the hydrophobic surface of CPE. Figure 8 shows the cyclic voltammograms of T_4 reduction at CPE in the presence of 5×10^{-6} M different surfactants. In the presence of CTAB, the reduction current is the highest and STAB takes the second place, followed by CPB. The presence of other cationic surfactants with shorter hydrophobic tails and the neutral surfactant, Triton X-100, produced neglectable effects on the reduction of T_4 . When an anionic surfactant, sodium dodecyl sulfonate (SDS), was added to the working solution, the reduction current was completely depressed. These results suggest that only the cationic surfactants with long hydrophobic tails can effectively improve the reduction of T_4 . Considering the strong interaction between iodine molecules and iodide ions, we suggest that bromide ions in cationic surfactants may form special ion complexes with T_4 through the interactions with the iodine atoms on T_4 , which can promote the

Fig. 8 Electrochemical responses of 5×10^{-6} M T_4 at CPE in 0.1 M HCl containing 6×10^{-5} M of stearyltrimethylammonium bromide (STAB), cetyltrimethylammonium bromide (CTAB), cetylpyridinium bromide (CPB), lauryltrimethylammonium bromide (LTAB), tetrabutylammonium bromide (TBAB), tetramethylammonium bromide (TMAB), Triton X-100, sodium dodecyl sulfonate (SDS) and no surfactant. Accumulation time, 5 min; accumulation potential, 0.9 V; scan rate, 100 mV/s.

reduction of T_4 . This explanation is consistent with the increasingly enhanced reduction of T_4 with the increase of the surfactant activity. The above assumption about the role of CTAB was also supported by the neglectable change of the electrochemical signals of T_4 at CPE with the addition of potassium bromide (KBr) (not shown). The effect of CTAB concentration on the peak current of R_1 was examined. The peak current increased with the increase of CTAB concentration when CTAB concentration was lower than 6×10^{-5} M, due to the adsorption of more CTAB molecules on the electrode surface. At the CTAB concentration of 6×10^{-5} M, the peak current of R_1 was the highest. With the continuous increase of CTAB concentration, the peak current dropped; this drop was attributed to the block of mass transfer of T_4 by the compact film of CTAB on the electrode surface. On the basis of the above discussions, it is reasonable to assume that the adsorption of CTAB on the hydrophobic surface of CPE may not only enhance the adsorption of T_4 at this surface by hydrophobic interaction but also may concentrate sufficient Br^- ions at the electrode surface for the inducement of T_4 reduction, which would account for the enhanced reduction of T_4 at CPE in the presence of trace CTAB.

Optimizing of some experimental conditions

Although the oxidation of OH on T_4 is sensitive for the detection, the interferences of this peak were serious because many compounds containing a phenol hydroxyl group may interfere with the electrochemical response. Thus, the enhancement effect for CTAB on T_4 reduction provides a potential and selective method for the detection of T_4 . To gain the best results, some experimental conditions for the reduction of T_4 were optimized.

The electrochemical responses of T_4 in HCl, HNO_3 and H_2SO_4 media were tested. It was found that 0.1 M HCl is a suitable electrolyte for the determination of T_4 . The effect of pH on the reduction of T_4 was estimated in the range of pH 1–6, and the reduction current decreased with the increasing of pH. The relationship between the peak potential and pH is complicated. With the increasing of pH, the peak potential positively shifted and reached a maximum at pH 4. Then it negatively shifted with the further increasing of pH. In the range of pH 1–3, the

peak potential (E_p) is linear with pH:

$$E_p = 0.029 + 0.3716\text{pH} \quad (R = 0.9933) \quad (2)$$

One can see from the above equation that the number of the electrons transferred in the reduction of T_4 is twice the number of protons, which is consistent with the proposed mechanism for the reduction of T_4 . The effect of scan rate (v) on the peak current was also investigated. The peak current linearly increased with the increase of scan rate in the range of 10 – 50 mV/s. It seems that the electrode process is controlled by adsorption, which is in accordance with the decreasing of the peak current of O_1 in Fig. 1 with the increasing of scan number. The relationship between the peak potential and scan rate can be described by the following equation:

$$E_p = -0.021\ln v + 0.532 \quad (3)$$

The reduction of T_4 is a totally irreversible process; thus the value calculated for αn_α from Eq. (3) is 0.84, where α is the transfer coefficient and n_α is the number of electrons transferred in the rate-determining step.

The accumulation potential is a major factor that affects the response sensitivity, since the reduction of T_4 is adsorption-controlled. In the potential range of 0.8 – 1.2 V, the peak current firstly increased with the increasing of the accumulation potential for an accumulation time of 5 min. Then the peak current reached a maximum value at about 0.9 V and fell down with the continuous increasing of the accumulation potential. Thus, an optimal accumulation potential of 0.9 V was selected in the sequential investigations.

Interferences

Trace substances commonly present in drugs were tested in order to check for interferences with respect to the responses. Interferent-to-analyst ratios in the range of 1:1 to 100:1 were examined for a T_4 concentration of 2.5×10^{-6} M. The interferences from two fold of estradiol, cholesterol, dopamine and uric acid, 100-fold of ascorbic acid, 40-fold of barbitone, are apparent, while the interference by two fold of estriol is serious. As for tyrosine, the interference is negligible. Comparing the structure of T_4 with these interferents, we conclude that the interferences caused by these interferents are due to their competitive combination with CTAB or their competitive occupation of active points on the electrode surface with T_4 . The high selectivity of the determination of T_4 arises from the specific reduction of iodine atoms on T_4 instead of from the oxidation of the OH group.

Calibration and application

The calibration curve has been established in the concentration range of 2×10^{-7} – 9×10^{-6} M, and the relationship between the concentration of T_4 and the peak current can be described with the following linear regression equations:

$$I_p = 0.286c + 0.316 \quad (R = 0.9968) \\ \text{(in the concentration range of } 4 \times 10^{-8} - 1 \times 10^{-7} \text{ M);} \quad (4)$$

$$I_p = 0.860c + 3.113 \quad (R = 0.9959) \\ \text{(in the concentration range of } 1 \times 10^{-7} - 1 \times 10^{-6} \text{ M);} \quad (5)$$

$$I_p = 5.736c + 7.696 \quad (R = 0.9877) \\ \text{(in the concentration range of } 1 \times 10^{-6} - 1 \times 10^{-5} \text{ M).} \quad (6)$$

Table 1 Data obtained from the determination of T_4 in a drug at CPE in 0.1 M HCl in the presence of 6×10^{-5} M CTAB by linear sweep voltammetry (LSV)

Drug sample	T_4 labeled ($\mu\text{g}/\text{tablet}$)	T_4 found ($\mu\text{g}/\text{tablet}$)	T_4 average ($\mu\text{g}/\text{tablet}$)	RSD, %
1	40	42.1	40.7	4.9
2	40	39.1		
3	40	38.5		
4	40	41.5		
5	40	42.3		

Concentration of thyroxine, 2.5×10^{-6} M; accumulation time, 5 min; accumulation potential, 0.9 V; potential scan range, 0.6 – 0.1 V; scan rate, 100 mV/s.

For an accumulation time of 5 min at 0.9 V, a detection limit of 6.5×10^{-9} M was obtained ($\sigma = 3$). The relative standard deviation was 4.1% for six parallel detections of 2.5×10^{-6} M T_4 at the same electrode, refreshed.

The method developed was employed for the determination of T_4 in drugs. The drugs were pretreated according to a previous report.⁷ Table 1 gives the results of T_4 detection in a kind of drug that was used for the treatment of hypothyroidism. This drug contains tissues of thyroid glands instead of thyroxine itself. In body liquid, thyroxine is released from this drug and exhibits its biological function. As can be seen from Table 1, good recoveries and the low relative standard deviation (RSD) are obtained. Thus, this method is sensitive and authentic for the detection of T_4 in drugs.

Conclusions

The electrochemical reduction of thyroxine (T_4) at carbon paste electrode (CPE) was investigated. The results showed that the reduction of T_4 was significantly improved in the presence of cetyltrimethylammonium bromide (CTAB). This was achieved through two important roles of CTAB: (i) the enhanced adsorption of T_4 by the hydrophobic interaction between T_4 and the adsorbed CTAB on the hydrophobic surface of CPE; (ii) the activation of iodine atoms on T_4 by the complexing interactions with bromide ions (Br^-) in CTAB that are accumulated near the electrode surface beforehand by the positively charged CTAB film on the electrode surface. The chronocoulometry studies showed that the reduction reaction occurred at the two iodine atoms on the phenol group of T_4 , producing two iodide ions. Compared with the oxidation reaction of T_4 , the reduction peak was more selective for the determination of T_4 while showing almost the same sensitivity. The interference and the calibration experiments showed that the media system developed in this work may be put into practice for the detection of T_4 .

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