Full Length Research Paper

Electrocatalytic determination of anti-hyperthyroid drug, methimazole, using a modified carbon-paste electrode

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Accepted 14 September, 2012

The preparation and electrochemical behavior of a carbon-paste electrode modified with an oxovanadium (IV) Schiff-base complex was studied. The modified electrode showed efficient catalysis in the electrochemical oxidation of methimazole (MMZ), an anti-hyperthyroid drug. The catalytic effect was observed for V^{III} oxidation peak which was produced via disproportionation of V^{IV} in acidic solution. The diffusion coefficient (D) of MMZ and the rate constant of catalytic reaction (k) were estimated by chronoamperometry. Determination of MMZ was carried out using differential pulse voltammetry. A linear dynamic range of 9×10^{-7} to 1×10^{-4} M with a detection limit of 8×10^{-7} M and a limit of quantitation of 2.6×10^{-6} M was resulted for the drug. The relative standard deviation for determination of 20 μ M of the drug was found to be 1.7%. The method was used for the quantitation of MMZ in pharmaceutical formulations successfully.

Key words: Methimazole, determination, tablets, modified-carbon paste electrode.

INTRODUCTION

Methimazole, MMZ (1-methyl-2-mercaptoimidazole, tapazole), is an orally taken drug used in the therapy of hyperthyroidism (over activity of the thyroid gland). The chemical structure of the drug is shown in Scheme 1. Its action is to slow down iodide integration into tyrosine and thus, inhibits the production of thyroid hormones (Edward, 1992). MMZ is used as a drug to manage hyperthyroidism associated with Graves' disease, but it has side effects such as possible decrease of white blood cells (Kendall-Taylor, 1984). The drug has also been employed to promote growth in animals for human consumption. It has been reported that MMZ may also cause side effects such as nephritis, liver cirrhosis, skin irritation, allergies and pharyngitis with fever (Edward, 1992).

Several analytical methods have been described for the determination of MMZ, such as high-performance liquid

chromatography (Blanchflower et al., 1997; Kusmierek and Bald, 2007), gas chromatography-mass spectrometry (Zhang et al., 2005), flow-injection analysis with chemiluminescence detection (Economou et al., 2004), spectrophotometry (Jovanovic et al., 1992), fluorescence probe method (Dong et al., 2009) and gold nanoparticle-catalyzed chemiluminescence reaction (Sheng et al., 2011). Electrochemical methods, due to their advantages such as high sensitivity, high speed, and low-cost have been used in pharmaceutical analysis for a long time. Analysis of MMZ has been done by using modified electrodes such as glassy carbon electrode modified with multi-walled carbon nanotubes (Xi et al., 2010), an electrode modified with acetylene black/chitosan film (Yazhen, 2011), and a carbon paste electrode modified with a cobalt Schiff base complex (Shahrokhian and Ghalkhani, 2008).

Compared to conventional electrodes, chemically modified electrodes offer unique well-recognized advantages in electrocatalysis, especially in situations where the target analyte requires high over-potential, or

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the sensitivity is low. This characteristic of chemically modified electrodes arises from the combination of conventional electrochemical techniques with the chemical, structural and other specific properties of the modifying layer(s).

Among the modified electrodes those which include inorganic complexes have been largely used in the area of electrocatalysis (Galal, 1998; Wu et al., 1996). Owing to the reversible redox activity and catalytic properties of vanadium-salen complexes, they have been used in electrocatalytic oxidation of dipyrone (Teixeira et al., 2004) and L-dopa (Teixeira et al., 2004). In the present work, the preparation and electrochemical properties of a carbon paste electrode modified with an oxovanadium (IV) Schiff base complex (VOx) for voltammetric determination of MMZ is discussed. Carbon-paste is a well-known substrate for incorporation of modifiers, as a simple and easy renewing material to construct a modified electrode (Kalcher et al., 1995).

The modified electrode showed good sensitivity in the determination of MMZ, and a long life time without significant decrease in its response. It was successfully applied to the determination of MMZ in tablets.

MATERIALS AND METHODS

Chemicals

The VOx (Scheme 1) was synthesized in Yasuj University, Yasuj, Iran. The procedures for preparation and structural identification of VOx were described in literature (Kianfar et al., 2011; Liu and Anson., 2001). Briefly, the vanadyl complex was synthesized by refluxing a methanolic solution of the Schiff base ligand and vanadylacetylacetonate. The reaction was continued for 2 h until a green precipitate was obtained. It was filtered, washed with methanol and dried in vacuum.

Pure powder of MMZ was a gift from Osvah Pharmaceutical Company (Tehran, Iran). Sodium hydrogenphosphate and hydrochloric acid (both from Fluka) were used to prepare the buffer solution. All other reagents were of analytical grade from Merck (Darmstadt, Germany) and were used without further purification. Doubly distilled water was used for preparation of aqueous solutions. A standard MMZ stock solution (1.0×10^{-3} M) was prepared from the pure powder and stored at 4°C. The working solutions were freshly prepared by serial dilution of the stock solution with 0.1 M phosphate buffer saline (PBS, pH 3).

Apparatus

The electrochemical measurements were performed using a Potentiostat/Galvanostat μ -Autolab system (Utrecht, The Netherlands). A three-electrode system, including a carbon paste working electrode, a platinum wire as the counter and a saturated calomel reference electrode (SCE) was employed. A Jenway 3345 pH/mV meter using a combined glass electrode was used for pH measurements.

Electrode preparation

The general procedure to prepare the modified carbon paste electrode was to mix VOx complex (0.2 g) with graphite powder (0.8 g)

Scheme 1. Chemical structures of MMZ (left) and the oxovanadium Schiff base complex (right).

and mineral oil (0.2 g). After thorough hand mixing using a mortar and pestle to obtain a very fine paste, the mixture was packed homogeneously in a glass tube. A copper wire was inserted into the carbon paste to implement the electrical conduction. The surface of the modified electrode (VOx/CPE) was finally smoothed manually on a clean filter paper. An unmodified carbon paste electrode was prepared using the same procedure in the absence of VOx complex.

Preparation of real samples

Ten tablets of MMZ (5 mg/tablet) were weighed and ground to a homogeneous powder. A proper portion of the fine powder (equivalent to 5 mg of MMZ) was carefully weighed and mixed with about 20 ml of PBS buffer (pH 3; 0.1 M). The suspension was stirred and heated for 10 min. and then filtered to remove insoluble residue. The clear solution obtained was diluted to 50 ml in a volumetric flask using the same buffer solution.

General voltammetric procedure

10 ml of the buffer solution (PBS; pH 3; 0.1 M) was transferred to the voltammetric cell. The electrodes were immersed in the solution and after setting the potential range and scan rate, the cyclic voltammogram was recorded. Then a proper portion of MMZ solution was added to the cell and the stated procedure was repeated.

Evaluation of limit of detection (LOD) and limit of quantitation (LOQ)

In order to evaluate LOD and LOQ, the voltammetric signal (anodic peak current) was obtained for the buffer solution repeatedly ($n = 10$) and the mean value (y_b) and standard deviation (s_b) of the results were obtained. LOD was calculated as a concentration of MMZ which its voltammetric signal was $y_b + 3s_b$. In the case of LOQ, the voltammetric signal should be $y_b + 10s_b$.

RESULTS AND DISCUSSION

Electrochemical behaviour of VOx complex

In order to study electrochemical behaviour of the vanadium complex and due to its insolubility in aqueous solution, the complex was dissolved in acetonitrile. Cyclic

voltammograms of VOx solution in acetonitrile were recorded at different scan rates (5 to 100 mV s⁻¹). The potential range of the working electrode was from 0.0 to 1.0 V against SCE. As shown in Figure 1, a well resolved pair of anodic and cathodic peaks appeared with a peak separation of about 0.07 V and $E_{1/2}$ [(E_{pc} + E_{pa})/2] of 0.06 V. The ratio of cathodic peak current (I_{pc}) to anodic peak current (I_{pa}) was about one in the range of scan rates used. These observations confirm a reversible electron transfer mechanism between two oxidation states of vanadium in the complex (Kianfar et al., 2011; Liu and Anson, 2001):

As shown in Figure 1, the effect of increasing scan rate is a gradual increase of both anodic and cathodic currents without potential shift which indicates a simple electron transfer mechanism. The anodic peak current increases linearly with the square root of scan rate (Inset), which shows the diffusional nature of the currents (Bard and Faulkner, 2001).

A carbon-paste was modified with VOx (VOx/CPE) and used as the working electrode in cyclic voltammetry. Figure 2 shows the result in aqueous solution (PBS, pH 3). The anodic peak (A_1) and its cathodic counterpart (C_1) observed at the surface of carbon-paste electrode are similar to those observed in acetonitrile solution which correspond to the V^V/V^{IV} couple. Another redox couple (A_2 and C_2) is observable at higher potentials (about 0.8 V) which is due to electron transfer of V^{III}/V^{IV} couple (Liu and Anson, 2000).

Vanadium (III) is formed in acidic solutions from V^{IV} through a disproportionation mechanism as shown in reaction b which is oxidized at the electrode surface:

Both reactions occurred at the surface of the electrode, while Reaction b occurred between the vanadium complex and the aqueous solution, the electron transfer Reaction c was conducted through the electrode. Due to the necessity of acidic conditions for disproportionation, the second pair (A_2 and C_2) was not observed in acetonitrile solution.

Electrocatalytic effect of VOx on the oxidation of MMZ

Figure 3 is a comparison between cyclic voltammograms of MMZ at the surface of an unmodified-carbon-paste electrode and VOx/CPE. As is obvious, in the presence of MMZ, the oxidation peak of V^{III} significantly increased which may be attributed to the catalytic effect on the oxidation of MMZ to the disulfide product (Aragoni et al.,

Scheme 2. Representation of electrocatalytic oxidation of MMZ.

Figure 1. Cyclic voltammograms of VOx complex (1.0×10^{-4} M) in acetonitrile on a glassy carbon electrode at various scan rates: from inner to outer, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mV s⁻¹. Supporting electrolyte: tetraethylammonium perchlorate (0.1 M).

Figure 2. Cyclic voltammogram of VOx/CPE in a PBS (pH 3, 0.1 M). Scan rate, 50 mV s⁻¹.

Figure 3. Cyclic voltammograms at a bare carbon-paste electrode (a) in the absence, (b) in the presence of 40 μM MMZ; and at VOx/CPE (c) in the absence and (d) in the presence of 40 μM MMZ; supporting electrolyte, PBS (pH 3.0, 0.1 M); scan rate, 50 mV s^{-1} .

2002). Scheme 2 shows the whole process at the surface of modified electrode in which a dimer is formed between two molecules of MMZ as the oxidation product.

Although MMZ was oxidizable at the surface of unmodified electrode (Figure 3) but the anodic current was much weaker than the electrocatalytic current at VOx/CPE.

The effect of pH of the solution on electrocatalytic peak current was studied. The anodic current decreased with increasing pH from 3.0 to 7.0 because protons are involved in the disproportionation reaction (Reaction b) to produce V^{III} . A pH of 3.0 was selected for the electrocatalytic oxidation of MMZ.

The effect of different scan rates (10 to 200 mV s^{-1}) on the electrocatalytic current was studied in PBS (pH 3.0) solution (Figure 4A). The results showed that the peak current increased linearly with the square root of scan rate (Figure 4B), which demonstrates that the anodic current is controlled by diffusion of MMZ to the electrode surface (Bard and Faulkner, 2001).

In order to obtain the diffusion coefficient of MMZ to the surface of VOx/CPE, static chronoamperometry was used. Figure 5A shows the chronoamperograms for four different concentrations of MMZ. Diffusion coefficient was obtained from the slopes of the linear plots of currents (I) against $t^{-1/2}$ (Figure 5B) according to Cottrell equation:

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

Where D and C^* are the diffusion coefficient and the bulk concentration of MMZ, respectively. A is the geometric area of the carbon-paste electrode, t is the time elapsed,

Figure 4. (A) Cyclic voltammetric responses at different scan rates using VOx/CPE as the working electrode. [MMZ] = 40 μM ; supporting electrolyte, phosphate buffer (pH 3.0, 0.1 M); Scan rates from down to top: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180 and 200 mV s^{-1} . (B), variation of the catalytic current versus the square root of scan rate.

and n is the number of electrons in the electrode process which is 1 in this case.

An average amount of $1.24 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$ for D was obtained (Figure 5C). The rate constant for the chemical reaction between MMZ and redox sites in VOx/CPE, k , can be evaluated by chronoamperometry according to equation 2 (Galus, 1976):

$$I_C / I_L = \pi^{1/2} (kC^* t)^{1/2} \quad (2)$$

Where I_C is the catalytic current in the presence of MMZ, I_L is the limited current in the absence of MMZ, and k is the catalytic rate constant ($\text{cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The calculated value of k was $1.13 \times 10^7 \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ using the slope of the plot of I_C / I_L against $t^{1/2}$ (Figure 5D). The large value of k indicates a fast electron transfer reaction between MMZ and V^{III} ions.

Analytical aspects

Determination of MMZ

It was observed that the amount of catalytic current in cyclic voltammetry was proportional to the MMZ concentration. Since differential pulse voltammetry (DPV) has a higher sensitivity, it was used for the determination of MMZ. Figure 6A shows DPVs for various concentrations of MMZ on the surface of VOx/CPE.

The voltammetric calibration curve for the determination of MMZ, shown in Figure 6B, has a linear range from 0.9 to 100 μM with a LOD of 0.8 μM and LOQ

Figure 5. (A) Chronoamperograms obtained for VOx/CPE in the absence and presence of 20, 40, 60 and 100 μM MMZ in 0.1 M phosphate buffer. B) Cottrell plots for 20, 40, 60, and 100 μM MMZ. C) Dependence of slopes of Cottrell plots on concentration of MMZ. D) I_C/I_L versus $t^{1/2}$.

Figure 6. A) Differential pulse voltammograms for different concentrations of MMZ at VOx/CPE. [methiamazole]: 0.9, 2.7, 4.4, 6.0, 7.6, 9.2, 17.4, 25.4, 41.1, 56.3, 71.1, 85.4, 99.2 and 112 μM in 0.1 M PBS (pH 3.0). B) Plot of electrocatalytic peak currents versus MMZ concentration.

of 2.63 μM . The slope and the correlation coefficient (r^2) for the least squares line were 0.1 $\mu\text{A}/\mu\text{M}$ and 0.997, respectively. The relative standard deviation (RSD) for four replicate measurements of 20 μM of MMZ under

optimum conditions was 1.76%. The results are comparable (Shahrokhian and Ghalkhani, 2008) or better than (Aslanoglu and Peker, 2003) the literature reports on the electrochemical determination of MMZ. The electrode could be used several times without significant loss of current response. Before each experiment the electrode was simply washed with distilled water.

Interferences

Under optimum conditions, the interference effect of some organic compounds which may be present in real samples of MMZ was studied. A concentration of 5 μM of MMZ was used and the suspicious compound was added. The tolerance limit of interferent was defined as an amount of it that retains the electrocatalytic current between 95 to 105% of its initial amount in the absence of interferent. The results show that 150-fold of amino acids asparagine, D, L-alanine, L-histidine and 40-fold glucose in the solution had almost no influence on the determination of MMZ.

Determination of MMZ in pharmaceutical preparations

In order to demonstrate the capability of VOx/CPE to the electrocatalytic quantitation of MMZ in real samples,

Table 1. Determination of MMZ in tablet samples.

Sample	MMZ (mol L ⁻¹)	Found (mol L ⁻¹)	Recovery %
1	3.8×10^{-6}	3.6×10^{-6}	95
2	5.7×10^{-6}	5.3×10^{-6}	93

tablets of MMZ (5 mg MMZ per tablet) were used as real samples. The calibration curve was plotted using the standard MMZ solutions and the amount of the drug in tablet was calculated by using the regression line equation. The results for the analysis of two different aliquots of the tablet solution are shown in Table 1. As it is shown, the recoveries are quite satisfactory and the modified electrode VOx/CPE can be successfully applied in the analysis of trace amounts of MMZ in pharmaceutical preparations.

Conclusions

In the present article, a VOx was used in the bulk-modification of a carbon-paste electrode. Cyclic voltammetry in PBS (pH 3) showed two redox couples for the vanadium complex, that is, V^{IV}/V^V and V^{III}/V^{IV} couples, in which V^{III} was produced by a disproportionation chemical reaction in acidic solution. The modifier showed an excellent electrocatalytic activity towards the oxidation of MMZ through the second redox couple, that is, V^{III}/V^{IV} . The diffusion coefficient of MMZ and the rate constant of the catalytic chemical reaction were calculated using chronoamperometry. DPV was used in the determination of MMZ in pharmaceutical preparations. The results were quite satisfactory. Ease of preparation of the modified electrode and the simple renewal procedure of it, as well as good reproducibility of the voltammetric responses make it an efficient sensor for the detection of trace amounts of MMZ in pharmaceutical samples.

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