ELECTROCHEMICAL DETERMINATION OF RIBOFLAVIN USING A SYNTHESIZED ETHYL [(METHYTHIO)CARBONOTHIOYL] GLYCINATE MONOLAYER MODIFIED GOLD ELECTRODE

© 2016   F. Karimian, G. H. Rounaghi[1], M. Mohadeszadeh

Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad
Mashhad, Iran

*[1]E-mail: ghrounaghi@yahoo.com; ronaghi@um.ac.ir*

The surface of a gold disk electrode, for the first time, was modified with a self-assembled mono layer of a synthesized compound, ethyl [(methythio)carbonothioyl] glycinate (**ECTG**), for construction of an electrode sensitive to riboflavin (vitamin B_2). The electrochemical properties of the monolayer assembled on the gold disk, were investigated by cyclic voltammetry and electrochemical impedance spectroscopy. Under the optimized conditions, the voltammetric peak currents resulting from vitamin B_2 (**VB_2**) species were linear for VB_2 concentrations in the range from 10^{-6} to 10^{-2} M. The effect of pH, type of buffer solution and scan rate on the response of the modified electrode was studied. The constructed electrochemical sensor responses very well to VB_2 in the presence of most common vitamins. Finally, the performance of the Au–ECTG modified electrode was successfully tested for electrochemical detection of VB_2 in a pharmaceutical sample.

Although typically techniques employed in clinical laboratories encompass titrimetry, chromatography, spectrophotometry and immunoassay, the use of electroanalytical interfacial techniques have attracted attention as accurate, sensitive and cost-effective methods of analysis [1]. In recent years, a great interest has been given to self-assembled monolayer (**SAM**) electrodes as tools for voltammetric determination of organic and inorganic compounds, in view of their advantages over ordinary electrodes: good reproducibility, easy preparation, exclusion of toxic components such as mercury, possibility of introducing on the electrode selective functional groups able to bind specific compounds [2–4]. Moreover, limitations that are often encountered in electrochemical determinations at thin polymeric film electrodes, typically slow diffusion across the film, are minimized in SAMs [5]. In particular, the development of SAMs based on chemisorption of thiols or disulfide on gold electrode surface, has greatly increased in the last years also for their stability [6, 7]. Such films are formed simply by dipping a cleaned gold electrode in ethanolic solution of the thio-compound of interest. The chemisorption of the −SH group onto the gold surface is followed by an ordering step in which the chains of the molecules co-align to form a highly ordered surface film.

Flavins are a class of organic compounds based on the cyclic base pteridine. Their core structure consists of the tricyclic heterocycle isoalloxazine. A flavin with significant biochemical importance is riboflavin (vitamin B_2, Scheme 1). Vitamin B_2 is a water-soluble biochemical molecule widely existing in food and pharmaceutical products. Riboflavin is the coenzyme involved in sugar, protein and fat metabolism, promoting growth and cell regeneration. VB acts as an intermediary in the transfer of electrons in biological redox reactions and has an important function in cell growth [8]. It can promote skin, nails, hair normal growth, and eliminate the mouth, lips, tongue inflammation, promote vision and reduce eye fatigue. At the same time, riboflavin is a kind of phototropic compound, photo taxis, and photodynamic therapy photosensitive agent [9–11]. Analytical methods for the determination of VB_2 need to be both selective and sensitive because of the presence of potential interference and low concentration of analytes. Many analytical methods for the determination of VB_2 have been reported, including spectrophotometry [12, 13], HPLC [14, 15], fluorescence spectrometry [16] and chemiluminescence [17].
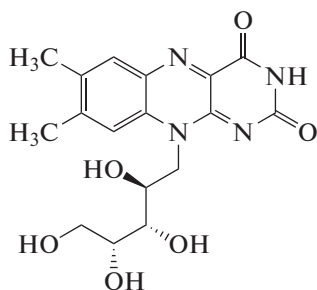
Scheme 1. Structure of vitamine B_2.

In the present study, we synthesized an organic compound and assembled it easily on the electrode surface as a modifier. To the best of our knowledge, ethyl [(methythio)carbonothioyl] glycinate has not been used as electrode modifier. We have studied the voltammetric response of an Au electrode modified with a self-assembled monolayer of ECTG for measurement of VB_2 over the concentration range of 10^{-6} to 10^{-2} M.
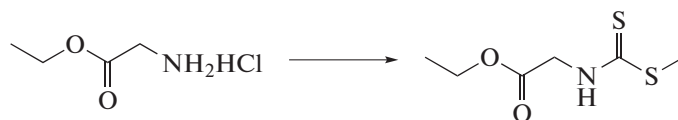
EXPERIMENTAL

Chemicals. Riboflavin, vitamins A, B_6, C, D_2 and E were obtained from the Sigma Chemical Company, while vitamin B_1 was obtained from Fluka. Sodium perchlorate, sodium acetate, acetic acid, potassium dihydrogen phosphate, potassium hydrogen phosphate and ethanol were purchased from Merck. Potassium ferricyanide, potassium ferrocyanide, sulfuric acid, sodium hydroxide, hydrochloric acid, chloroform, carbon disulfide, triethylamine, dimethyl sulphate and glycine ethyl ester hydrochloride were purchased from Sigma. All the drug samples tested were fresh and purchased from a local pharmacy. The riboflavin solutions were used immediately after preparation. All solutions were prepared by double-distilled water. Phosphate buffer solutions (**PBS**) in the pH range of 2.0–8.0 were prepared by 0.05 M KH_2PO_4 and

0.05 M K_2HPO_4, and the pH was adjusted by 0.1 M NaOH or HCl. The supporting electrolyte was 0.1 M $NaClO_4$. Nitrogen gas was passed through the solutions for de-aeration for 10 min prior to measurements.

Apparatus. All electrochemical experiments were performed using a µAutolab electrochemical system (Eco-Chemie, Utrecht, The Netherlands) equipped with a NOVA software (Eco-Chemie, Utrecht, The Netherlands). A three-electrode cell was employed in the experiments with a home-made disk gold electrode of diameter 0.01 cm as working electrode. The counter electrode was a platinum bar, and the reference electrode was a KCl-saturated Ag/AgCl. A locally made three-electrode glass cell with capacity of about 10 mL was used through the study.

Electrode modification. Prior to modification, the Au electrode was polished, first mechanically with alumina powder slurry (0.3 down to 0.05 mm, Buehler) on a polish cloth, sonicated successively in water/chloroform/water baths, each step 5.0 min, to remove any physically adsorbed species; and then, electrochemically by cycling the electrode potential from 0 to 1.5 V vs. Ag/AgCl electrode in 1.0 M H_2SO_4 until reproducible cyclic voltammograms were obtained. SAM were prepared by dipping the cleaned gold electrode in 5 mM ethanolic solution of ECTG for 24 h at room temperature, and then the electrode was rinsed with ethanol to remove the physically adsorbed ECTG.

Synthesis of ECTG. Scheme 2 shows the synthesis of ECTG. Glycine ethyl ester hydrochloride (1.4 g, 10 mmol), CS_2 (0.76 g, 10 mmol) and triethylamine (1.96 g, 20 mmol) were reacted at 40°C in chloroform (20 mL) for 1 h. Then dimethylsulphate $(CH_3O)_2SO_2$ (1.26 g, 10 mmol) was added drop-wise during 5 min and the mixture was heated under reflux for 2 h. The solution was cooled, washed with water (10 mL), and the organic layer was evaporated. Then the precipitate was filtered and washed with water (5 mL).


Scheme 2. Synthesis of ECTG.

Electrochemical measurements. The electrochemical experiments were carried out in a three-electrode conventional cell including the gold disk as a working electrode, a Pt wire as an auxiliary electrode, and an Ag/AgCl electrode as a reference. All reported potentials are referenced to the Ag/AgCl electrode. The electrochemical cell was placed in a Faraday cage to eliminate any environmental stray effect. The measurements were carried out under room temperature.

RESULTS AND DISCUSSION

Characterization of gold electrode modified with ECTG layer. The electrochemical characteristics of ECTG SAM were studied by electrochemical probe and electrochemical impedance spectroscopy (**EIS**). The electrochemical properties of ECTG SAM on gold electrode were studied by cyclic voltammetry using the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ system as a redox probe. Fig. 1 shows the cyclic voltammetric responses of the
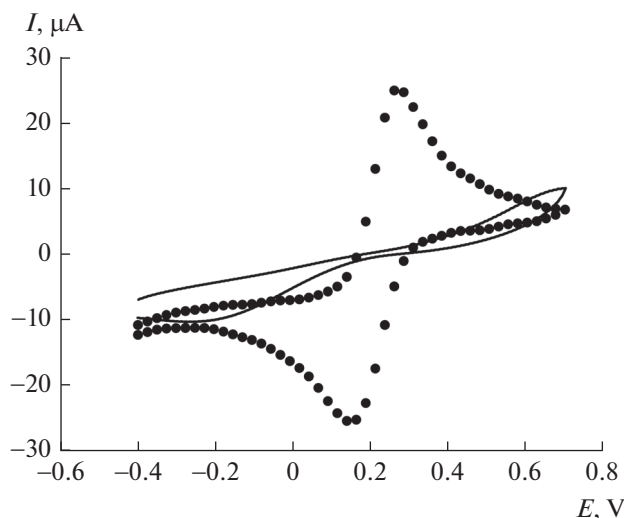
Fig. 1. Cyclic voltammograms of 5 mM $Fe(CN)_6^{3-/4-}$ at the bare (−) and ECTG SAM modified (•) gold electrode.

bare and ECTG SAM modified gold electrodes in the presence of $Fe(CN)_6^{3-/4-}$. In the case of the bare gold electrode, a pair of easily reversible waves with a small peak potential separation of 0.01 V was observed at a scan rate of 0.10 V/s. Upon the monolayer formation on the gold electrode surface, we observed a quick decrease in the current response and very weak redox peaks were obtained at the ECTG monolayer modified electrode in the potential range from −0.40 to 0.70 V, indicating that the SAMs inhibit the electron-transfer process between the probe and the gold electrode.

It is well known that the electrochemical alternating current impedance technique is a useful tool for studying the interface properties of surface-modified electrodes [18−21]. Therefore, EIS measurements were also used to characterize the ECTG monolayer. The EIS is a powerful, nondestructive, and informative technique to examine an electrochemical system, in which the interfacial impedance of the electrode/solution is influenced by specific microscopic events of interest. Some parameters such as solution resistance (R_s), charge transfer resistance (R_{ct}), apparent charge transfer rate constant (k_{app}), and the double layer capacitance (C_{dl}) may be evaluated from the EIS measurements which is attributed to the electrode/solution interface [22, 23].

Here, the fabrication steps of Au−ECTG electrode were more precisely traced by the EIS measurements. The complex impedance plane (Nyquist) plots obtained for the bare Au and Au−ECTG SAM electrodes in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ in the test solutions at pH 8.0 which are displayed in Fig. 2. Comparison of the complex impedance plots of the bare gold electrode and the monolayer-covered gold
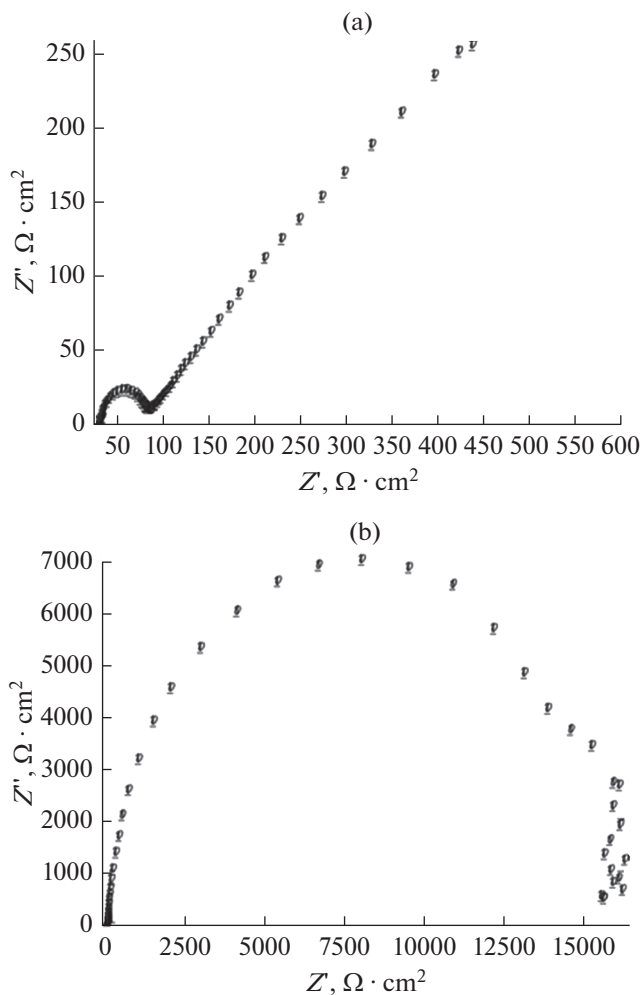
Fig. 2. Complex plane plots obtained for faradaic impedance measurements in PBS containing 0.1 M $NaClO_4$ in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ at pH 8.0 on bare electrode (a) and modified gold electrode (b).

electrode shows the effect of the adsorbed ECTG monolayer on the AC response. For the monolayer-covered electrode, the R_{ct}, which is the diameter of the semicircle at high frequency, is clearly greater than that of the bare gold electrode due to inhibition of the ECTG SAM to electron transfer. This also proves the presence of ECTG on the gold electrode.
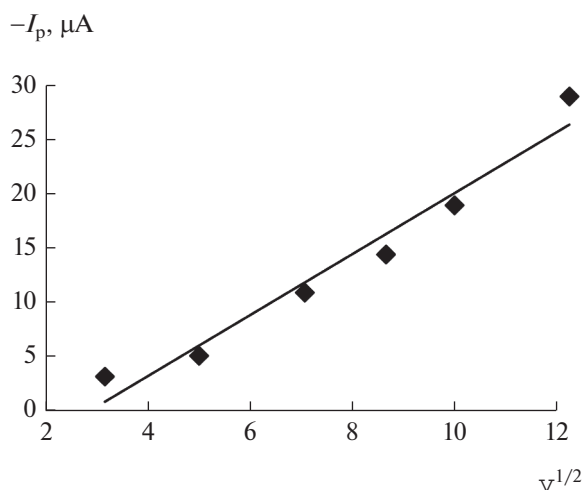
VB$_2$ electrochemistry at SAM electrode. Fig. 3 presents the cyclic voltammogram for a 10^{-5} M solution of VB$_2$ which was obtained with the Au−ECTG electrode. In the presence of VB$_2$, a reversible waves is observed at 0.4 V. The peaks currents results from the oxidation and reduction processes of VB$_2$ because the Au−ECTG electrode does not exhibit any current wave for blank solution under the same experimental conditions. It can be seen that a current peak is appeared at 0.4 V at the bare gold electrode in 10^{-5} M solution of VB$_2$. Comparison of the curves in Fig. 3 re-
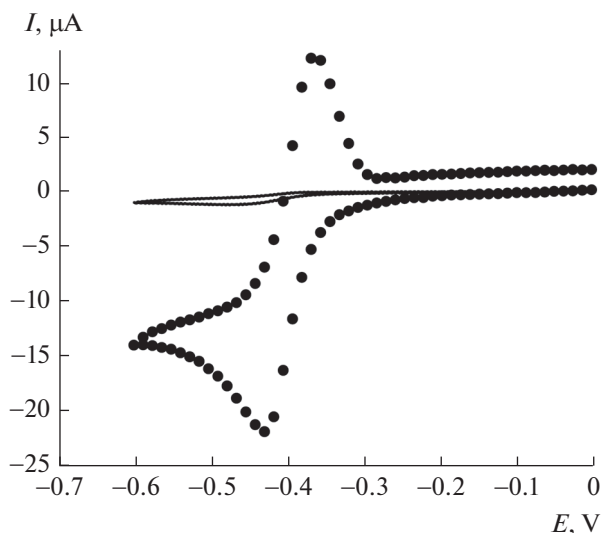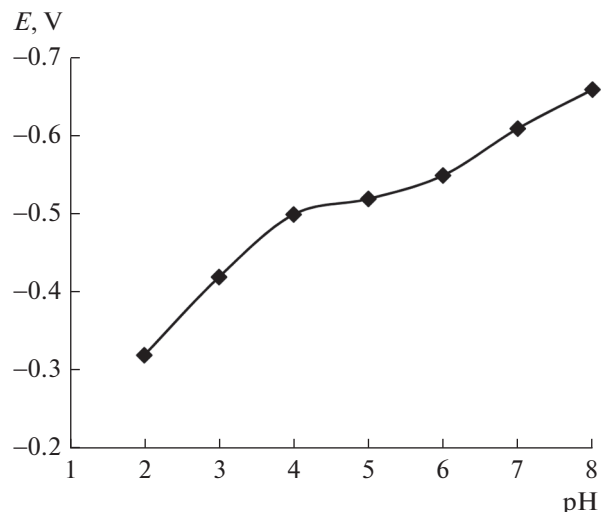
Fig. 3. Cyclic voltamogram of 10^{-5} M VB$_2$ solution at ECTG SAM modified gold electrode in Britton−Robinson buffer (pH 3) at a scan rate of 50 mV/s: (•) − modified, (−) − bare electrode .

Fig. 5. The effect of pH on electrooxidation potential of 10^{-5} M VB$_2$ using ECTG SAM modified gold electrode.

veals that the peak current increases when the Au−ECTG electrode is used. Therefore, the voltammetric response of the Au−ECTG electrode to VB$_2$ is attributed to the differences in the surface properties of electrode and the adsorption interactions between the analyte and the electrode surface.

Optimization of the analytical conditions. *Effect of scan rate on the voltammetric response of VB$_2$.* The influence of the scan rate varying from 10 to 150 mV/s on the voltammetric response of the modified electrode was studied. The cyclic voltammograms of the modified electrode indicate that the ratio of the anodic to cathodic peak currents which are obtained at various

scan rates decreases but the separation between the anodic and cathodic peaks (ΔE_p) increases. As is evident from Fig. 4, there is a linear relationship between the $v^{1/2}$ and the peak currents. This behavior indicates that oxidation is controlled by diffusion.

Influence of pH and buffer solution. In the determination of organic substances, the pH of the medium is an important factor affecting both the rate of the electrode process and its mechanism. The voltammetric behavior of VB$_2$ was examined at different pH using cyclic voltammetry. The pH dependence of the potential peak of vitamin B$_2$ is complex. Fig. 5 shows the electrooxidation potential of typical cyclic voltammograms of 10^{-5} M VB$_2$ in Britton−Robinson buffer solutions with pH values ranging from 2 to 8 at scan rate 100 mV/s using Au−ECTG electrode. As is evident, the peak potential is shifted to the cathodic region, which shows that the electrooxidation of VB$_2$ is facilitated by the stepwise deprotonation of its different forms. The deprotonation may precede the stage of the electron transfer or occur simultaneously with it.

Furthermore, different supporting electrolytes such as phosphate buffers solution, NaOAc−HOAc and NH$_3$−NH$_4$Cl were used in these experiments and it was found that the electrochemical response of VB$_2$ was best in the case of using PBS buffer solution.

Calibration curve and the effect of interferences. Under optimized conditions for the cyclic voltammetric experiments, the calibration curve was obtained at pH 2.0 using a PBS. The dynamic range of the calibration curve is between 10^{-6} and 10^{-2} M of VB$_2$. The equation of line and correlation coefficient are:

$$y = 5.32x - 8.92, \quad R^2 = 0.9985.$$

Fig. 4. The effect of scan rate on peak current of 10^{-5} M VB$_2$ at ECTG SAM modified gold electrode.

Table 1. The peak current for reduction of VB₂ in the presence of other vitamin

Interfering species	Concentration ratio VB₂ : interference	Anodic peak current of 10 μM VB₂, μA
Vitamin A	1 : 20	17.4
Vitamin B₁	1 : 100	18.1
Vitamin B₆	1 : 100	16.9
Vitamin B₁₂	10 : 1	9.3
Vitamin C	1 : 100	17.9
Vitamin D₂	1 : 100	16.1

Table 2. The analytical results obtained for determination of VB₂ tablets

Lable amount, mg	Found, mg	RSD, %	Recovery, %
5.0	4.89	0.30	98.5
5.0	5.12	0.71	100.4
5.0	5.07	0.56	97.0

The effect of interferences due to the presence of the other vitamins which are the major components of multi vitamin pharmaceutical preparations, was studied under the same experimental conditions. The interference studies were conducted by exposing the modified electrode to a solution containing 10 μM VB₂ and a specific concentration of the interfering molecule. Voltammetric responses resulting from the presence of interfering molecules were compared with that obtained for pureVB₂ incorporation. The experimental results are given in Table 1. As is obvious, most of the other studied vitamins did not show a significant interference when present in ten-fold excess with respect to VB₂ in solution. But the VB₁₂ showed a significant interference even at its very low concentration. The obtained results show that the selective determination of VB₂ species could be attained in the presence of most common vitamins in solutions.

Analytical application. The VB₂ content of some tablets was determined from calibration and the results are listed in Table 2. The relative standard deviation was found to be 0.30–0.71%, which indicates the applicability of the proposed method for quantitative determination of the VB₂ in real samples. Four parallel measurements were made everywhere. Further, in order to establish the suitability of the proposed method, known amounts of the standard VB₂ were added into the analytical solutions of the VB₂ tablets and the same procedure was applied for measurement of this vitamin in solution. The recoveries indicate that the accuracy and repeatability of the proposed voltammetric method are very good. The results obtained for the recovery of VB₂ (Table 2) agree well within the experimental errors.

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Synthesized ethyl [(methythio)carbonothioyl] glycinate was used for modification of an Au electrode. Electrochemical properties of the self-assembled monomolecular layer of ECTG were investigated by cyclic voltammetry and electrochemical impedance spectroscopy. The constructed Au electrode which was modified with a self-assembled monolayer of ECTG was used as a vitamin B₂ sensor. This electrode exhibited a relatively selective voltammetric response to VB₂ under the optimized experimental conditions (scan rate 0.1 V/s, pH 2 and PBS buffer solution), over the concentration range of 10^{-6} to 10^{-2} M. The electrode was successfully applied for the determination of riboflavin in pharmaceutical preparation samples. The results also showed that simultaneous determination of riboflavin in the presence of some other vitamins is possible. The constructed electrochemical sensor has a high precision, good sensitivity, simplicity, inexpensive and rapid response for determination of concentration of VB₂ in solutions. The proposed electrode in comparison with the best electrodes of this kind reported earlier, is superior with regard to the simplicity method of modification and analysis, ordinary agents used for film-coating, uncomplicated evaluation of electrochemical properties of the monolayer, working concentration range and absence of interference of other common analytes.

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