Modified Glassy Carbon Electrode를 이용한 Thiamine의 전기화학적 정량

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Electrochemical method for determination of thiamine using modified glassy carbon electrode

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Abstract

Glassy carbon electrode (GCE) modified with poly(4-((2-pyridylazo)-resorcinal) (PAR) film was used for the determination of thiamine. Thiamine was accumulated at the PAR modified GCE surface under open circuit condition. Thiamine gives sensitive oxidation peaks at 160 mV upon using the differential pulse voltammetry (DPV) technique. Experimental conditions such as supporting electrolyte, pH value and accumulation time were optimized for the investigated method. Thiamine gives linear response over the range of 0.030 mM to 1.1 mM. The lower detection limits were found to be 0.008 mM for thiamine. The investigated electrochemical method was successfully applied for the determination thiamine in pharmaceutical formulation.

Keywords: Thiamine, electrochemical method, poly(4-((2-pyridylazo)-resorcinal), glassy carbon electrode

1. Introduction

The chemical name for thiamine is 2-[3-[(4-amino-2-methyl- pyrimidin-5-yl)methyl]-4-methyl-thiazol-5-yl] ethanol which consists of a pyrimidine- and a thiazole moiety linked by a methylene bridge. Vitamin B₁ is necessary for the proper functioning of the nervous (1) and cardiovascular (2) systems of the body. Severe dietary deficiency of vitamin B₁ (vitamin B₁) can result in beriberi or the Wernicke-Korsakoff syndrome (3). Scientific literature reports many methods for the determination of thiamine, e.g. titrimetric (4), electrochemical analysis (5), gas chromatography (6), high performance liquid chromatography (7), and spectrophotometry (8) etc. Poly (4-((2-pyridylazo)-resorcinal) (PAR) modified electrode has been reported for the electrochemical determination of various compounds (9,10). In the current study, PAR polymer film modified GCE is described for the determination of
thiamine. The proposed method possesses many advantages such as low detection limit, fast response, simple and inexpensive instrumentation.

2. Experimental

Reagents and solutions

All reagents were of analytical grade. Thiamine (Sigma, USA) was used as standard. Stock solution (1 x 10^-2 M) and working standard of thiamine was prepared in distilled water. Poly (4-(2-pyridylazo)resorcinol) (PAR) was purchased from Aldrich (USA). 1 mM PAR solution was prepared by dissolving appropriate amount in pH 5 phosphate buffer solution. NaH_2PO_4.H_2O (Merck, Germany) and Na_2HPO_4.2H_2O (Merck, Germany) were used for phosphate buffer solution. The sample solutions were prepared by dissolving the tablets with water and diluted to the desired concentration level in the calibration range.

Apparatus

All electrochemical measurements were carried out using computer-controlled portable Potentiostat (RS-PDA1, Palm Instruments, Netherlands). A conventional three-electrode system was used throughout the experiments. The working electrode was a bare or PAR modified GCE (3.0 mm in diameter). The auxiliary electrode was a platinum wire and an Ag/AgCl electrode was used as a reference. pH meter (Mettler Toledo MP220, UK) was used for pH measurement.

Preparation of poly PAR modified GCE

The GCE surface was initially polished with fine emery paper followed by 0.3 μm and 0.05 μm alumina powder and finally sonicated with Milli-Q water for 3–5 minutes. The GCE was modified as reported previously [9, 10]. Before electropolymerization the polished electrode was electrochemically pretreated in 0.1 M PBS pH 5.0 by scanning 20 times between electrode potential of -0.5 V and 1.8 V at scan rate of 100 mV/s. The PAR film was deposited on the pretreated surface by scanning 20 times of the corresponding monomer in 1 mM PAR under the same conditions as those in the pretreated procedure. The film deposited electrode was washed with distilled water.

General procedure

The PAR-coated GCE was first activated in pH 6 phosphate buffers by cyclic voltammetric sweeps between 0.0 and 0.6 V until stable cyclic voltammograms were obtained. After obtaining the stable cyclic voltammogram the PAR-coated GCE was transferred into new cell containing pH 6 phosphate buffer and certain concentration of analyte. After 1 minute of open-circuit accumulation, the differential pulse voltammogram from -0.2 to +0.7 V at a scan rate 40 mV/s was recorded for the analyte. Upon using the DPV technique the peak position for BZ and LD appeared at 160 mV. After every measurement, the PAR-modified GCE was dipped into 0.1 M NaOH solution for few seconds to remove the adsorbed substances. For reproducibility of the PAR-modified GCE surface before
each experiment 5 successive cyclic voltammetric sweeps was done in pure phosphate buffer (pH 6).

3. Results and discussion

**Electrochemical behavior of thiamine on PAR modified GCE.**

Initially the cyclic voltammogram was investigated for the poly-PAR modified GCE in pure phosphate buffer pH 6. Within the potential window from - 0.2 to +0.7 V, no observable redox peaks appeared. However, a well-shaped and highly sensitive oxidation peak appears at 160 mV after adding 100 mM of thiamine. On the reverse potential scan from - 0.2 to +0.7 V, there was no corresponding reduction peak, suggesting that the electrode reaction of thiamine at the poly-PAR modified electrode is totally irreversible.

The oxidation peak current for thiamine in the second cyclic voltammetric sweep decreases as compared to the first peak, indicating the adsorption of thiamine on PAR film. The electrode surface was cleaned by washing with distilled water followed by dipping for few seconds in 0.1 M NaOH solution to remove the adsorbed substances for reproducible results.

**Parameter optimization for thiamine at PAR modified electrode**

Accumulation time in the range of 0 to 2 minutes was optimized for thiamine and accumulation of thiamine at the modified GCE was performed under open-circuit condition. The optimum scan rate for CV and DPV was 100 and 40 mV/s respectively. The best oxidation response was obtained in pH 6 phosphate buffer since the peak current is the highest and the peak shape is well-defined.

**Calibration curve**

DPV technique was applied to real sample due to good discrimination against background current and low detection limits. The linear increase in the current response for concentration of thiamine using DPV is shown in fig 1. Using optimum conditions linear calibration curves were obtained for thiamine over the range of 0.030 mM to 1.1 mM. The lower detection limits were found to be 0.008 mM for thiamine. Similarly calculated limit of quantification were 0.02 mM.

**Determination of thiamine in pharmaceutical preparation.**

The proposed electrochemical method was applied to the determination of vitamin B₃ in commercial vitamin B₃ tablets (Biphane, Yuhan, Co. Korea). Standard calibration curve was used for the determination of vitamin B₃. The summarized results for the analysis of thiamine in pharmaceutical preparations are shown in table-1. The results show that the proposed method can be successfully applied for the determination of thiamine in pharmaceutical preparation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Claimed/tablet</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biphane (vitamin B₃ tablet)</td>
<td>8.0 mg</td>
<td>8.34 mg</td>
</tr>
</tbody>
</table>

**Table 1.** Assay of vitamin B₃ in the commercial vitamin B₃ tablets
4. Conclusion

An electrochemical method was developed and successfully applied for the determination of thiamine using poly(4-(2-pyridylazo)-resorcinol) (PAR) modified glassy carbon electrode. In the anodic sweep from −0.2 to 0.7 V, thiamine adsorbed at the PAR polymer film modified electrode surface and oxidized at 160 mV using DPV method. The investigated electrochemical method is simple, sensitive, easy to apply and economical for routine analysis. The developed method was applied for the determination of thiamine using DPV method in pharmaceutical formulations.

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References