Electrochemical quantification of vitamin B\textsubscript{9} on poly tyrosine modified pencil graphite electrode

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Abstract: An electrochemically polymerized tyrosine film on pencil graphite substrate was found to be an affordable electrochemical sensor for vitamin B\textsubscript{9} or folic acid. The electrochemical characteristics of the electrode was studied using cyclic and differential pulse voltammetric techniques in phosphate buffer (pH 7). The surface study of the electrode was carried out by scanning electron microscopy. The sensor showed a linear range from 1µM – 85 µM concentration range by means of differential pulse voltammetry. A good repeatability was obtained for the developed sensor and was utilized for the sustainable application in pharmaceutical tablets.

1. INTRODUCTION

Electropolymerization technique plays a prominent role in providing polymer materials of considerable importance in various fields including electro-optical devices, battery applications and sensors [1]. Amino acids were widely used as monomers for the conducting polymers on different electrode substrates for electrochemical sensing applications in recent years [2]. Tyrosine is an important amino acid produced in our body, which helps the nerve cell communication by producing some chemicals in the brain. Here, we utilized a pencil graphite electrode (PGE) modified with poly tyrosine for the electrochemical sensing of folic acid. Folic acid (FA) or vitamin B\textsubscript{9} is one of the extremely important B vitamins used for the protection of unborn babies from serious birth defects [3]. The FA deficiency may be associated with patients having anemia, rheumatoid arthritis and even deficiency of FA decreases the blood serum level of vitamin B\textsubscript{12}[4]. Numerous analytical techniques including chromatography [5], spectrophotometry [6] and electrochemical methods [7,8,9] has been reported for FA determination. Now a days, pencil graphite electrodes are extensively used for the fabrication of electrochemical sensors for pharmaceuticals and biomolecules [10,11]. To the best of our knowledge, electrochemical sensor based on poly tyrosine modified pencil graphite electrode was hardly reported for folic acid quantification. Henceforth we developed an affordable electrochemical sensor with tyrosine modified PGE for the FA determination from commercially available folate tablets.

2. EXPERIMENTAL PART

2.1. Reagents and Instrumentation
Tyrosine and FA were obtained from LobaChemie Pvt. Ltd. All reagents and chemicals used were of analytical grade. Doubly distilled water was used to prepare entire solutions for our work. NaOH, Na\textsubscript{2}H\textsubscript{2}PO\textsubscript{4} and Na\textsubscript{2}HPO\textsubscript{4} used to prepare phosphate buffer solutions (PBS) were purchased from
Merck. The tablet Folvite-5 was purchased from a local pharma store. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI 610E (CH Instruments, USA) electrochemical workstation. Three electrode system was used with a platinum wire as the auxiliary electrode and the poly tyrosine modified PGE (diameter 0.7mm) as the working electrode. The PGE was covered with a Teflon tape exposing a length of 0.4 cm as the working surface. All potentials measured in this work were versus Ag/AgCl electrode (1M KCl).

2.2. Fabrication of electrode and analytical measurements
In a potential window of -0.8 to 1.8V, electropolymerisation of 1 mM tyrosine in 0.1M PBS 6 was done on a PGE with a scan rate of 100 mV/s for 20 segments. Then the modified electrode was washed with doubly distilled water and used for electrochemical measurements. The CV and DPV measurements were done in a range of -0.8 to 0.8 V and -0.9 to 0.4 V respectively.

2.3. Sample preparation
A 1mM solution of FA was prepared in 0.01M NaOH solution. For real sample solution, the FA tablet of the brand Folvite -5 was crushed homogeneously (30 tablets) and 1mM solution was prepared same as above. The solution was sonicated for 10 minutes for complete dissolution of folate content, which is filtered using ordinary filter paper and used for further analysis.

3. RESULTS AND DISCUSSIONS

3.1. Morphological description of the modified electrode
The surface study of the unmodified and the modified PGE was done with scanning electron microscopy (SEM) and is shown in figure 1A and 1B respectively. The SEM micrograph of unmodified PGE shows a regular flake like structure of graphite whereas the figure 1B shows an irregular surface of electropolymerized film of tyrosine on the PGE.

3.2. Electrochemical behaviour of FA on modified electrode
CV was primarily used for studying the electrochemical oxidation of FA. Figure 2 shows the CV of 75 µM FA on poly tyrosine modified PGE and bare PGE in 0.1 M PBS(pH 7) with a sweep rate of 200 mV/s. As seen in the figure, an enhancement in the peak current was observed in modified PGE compared to bare PGE for the electrochemical oxidation of FA. In the case of unmodified PGE, no oxidation peak was obtained for FA in the 0.1M PBS with pH 7. However, the CV of the modified PGE shows two oxidation peaks obtained for FA at -0.49 V and -0.68 V. For further quantification of FA, DPV studies were used in a potential range of -0.9 to 0.4 V. Two oxidation peaks were obtained at -0.72 V and -0.54 V in DPV in the above potential window. Later, the anodic peak at -0.72 V was utilized for calibration studies and analytical application of FA, as the peak at -0.72 V was more prominent at lower concentrations of FA. Further, the effect of scan rate (v) on the electro oxidation of FA was studied from 200mV/s to 500 mV/s (figure 3A) using CV. The plot of scan rate versus peak current shows a linearity with $R^2 = 0.99038$ suggests that the electrode process is adsorption controlled in nature (figure 3B).
3.3. Optimisation studies

The experimental variables such as pH of the supporting electrolyte, monomer concentration and polymerization cycles were studied to obtain maximum signal current during the electro oxidation of FA. DPV response of the 10µMFA in PBS with different pH were measured. Then the peak current attained a maximum value for PBS with pH 7, in comparison to acidic and basic pH and the data is given in table 1. Besides, a best analytical signal for FA was attained for the polymerization of PGE with 10 cycles (figure 4) in the optimized pH. Also, the effect of concentration of monomer on the DPV response of FA was studied using three different concentrations of tyrosine such as 0.5mM, 1mM and 1.5 mM. Then 1mM concentration was selected, as this concentration showed a better response compared to the others. Thus, the above variables which showed improvement in electrochemical response of FA were applied for the complete studies.

Figure 2. CV of 75 µM FA on (a) and (b) bare PGE without and with FA (c) and (d) poly -tyrosine modified PGE without and with FA in PBS pH 7 Scan rate: 200 mV/s

Figure 3. (A) CV curves of 75µM FA in 0.1M PBS 7 with different scan rates (B) Plot of scan rate versus peak current

Figure 4. Effect of polymerization cycles on peak current
3.4. DPV response of FA and repeatability of the electrode

The DPV characterization of FA was done with PBS 7 and two oxidation peaks were obtained at potential -0.72 V and -0.54 V in a potential range of -0.9 V to 0.4 V (figure 5A). The oxidation peak obtained at -0.72 V was used for the calibration studies. The electrode shows linear response for the FA determination from 1µM – 85 µM with R² = 0.99247 (figure 5B). The electrochemical response of three different electrodes towards 10 µM FA were monitored and a signal change with an RSD of only 1.37 % was observed. This showed the electrochemical response of FA within the working concentration range is highly reproducible on poly tyrosine modified PGE.

![Figure 5 - (A) DPV of FA in 0.1M PBS with pH 7 (B) Calibration graph](image-url)

3.5. Electrochemical detection of FA from folvite tablets

The analysis of FA from the solutions of folvite tablets were done by DPV and the data is given in table 2. An error of 9.7 % with the added amount and observed amount suggested that the poly tyrosine modified PGE works well for the pharmaceutical application of folic acid. Also, the developed sensor was compared with some of the existing reports for the electrochemical detection of FA and is given in table 3.

| Tablet  | Added (µM) | Determined (µM) | Percentage error |
|---------|------------|----------------|------------------|
| Folvite | 5          | 5.489          | 9.7 %            |

Table 3. Comparative study of our work with a few literature reports for FA determination

| Electrode | Concentration range (µM) | Supporting electrolyte | Reference |
|-----------|---------------------------|------------------------|-----------|
| Pd-Ag-co-doped SnO2/Pt | 22-112 | PBS pH 7 | 7 |
| AuCNS/AGR/MWCNT/GCE | 10 - 170 | PBS pH 7 | 12 |
| Methylene blue/rGO | 200 - 2300 | PBS pH 8 | 13 |
| Ni-POA/CPE | 100 - 5000 | 0.1M NaOH | 8 |
| ZrO₂ – CPE | 20- 2500 | PBS pH 7 | 9 |
| MWCNTs -PBCB | 900- 2310 | PBS PH 7 | 14 |
| Poly-Tyrosine- PGE | 1 - 85 | PBS pH 7 | This work |
4. CONCLUSION

A highly affordable and sustainable electrochemical sensor for one of the B vitamin folic acid was demonstrated. For the sensor fabrication a pencil graphite modified with poly tyrosine was used. The modified electrode showed a repeatability towards FA with an RSD of 1.37%. The quantitative application of the sensor was done by the effective determination of folic acid from the pharmaceutical tablet, folvite with an error of 9.7%.

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