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Selective electrochemical sensor for folic acid at physiological pH using ultrathin electropolymerized film of functionalized thiadiazole modified glassy carbon electrode

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ABSTRACT

This paper demonstrated the selective determination of folic acid (FA) in the presence of important physiological interferents, ascorbic acid (AA) and uric acid (UA) at physiological pH using electropolymerized film of 5-amino-2-mercapto-1,3,4-thiadiazole (p-AMT) modified glassy carbon (GC) electrode. Bare GC electrode fails to determine the concentration of FA in the presence of AA and UA due to the surface fouling caused by the oxidized products of AA and FA. However, the p-AMT film modified electrode not only separates the voltammetric signals of AA, UA and FA with potential differences of 170 and 410 mV between AA–UA and UA–FA, respectively but also shows higher oxidation current for these analytes. The p-AMT film modified electrode displays an excellent selectivity towards the determination of FA even in the presence of 200-fold AA and 100-fold UA. Using amperometric method, we achieved the lowest detection of 75 nM UA and 100 nM each AA and FA. The amperometric current response was increased linearly with increasing FA concentration in the range of 1.0×10^{-7}–8.0×10^{-4} M and the detection limit was found to be 2.3×10^{-10} M (S/N = 3). The practical application of the present modified electrode was successfully demonstrated by determining the concentration of FA in human blood serum samples.

1. Introduction

Folic acid (FA) is a significant component of the haematopoietic system and is the coenzyme that controls the generation of ferrohaeme (Merck, 1996). The decrease in concentration of FA in our body fluids leads to several complications including gigantocytic anaemia, leucopoenia, devolution of mentality, psychosis and increasing possibility of heart attack and stroke (Wei et al., 2006). It has also been suggest that decreased folate concentration is associated with enhanced carcinogenesis as folic acid with vitamin B_{12} participates in the nucleotide synthesis, cell division and gene expression (Gall and Van den Berg, 1993). Periconceptual supplementation of FA has been demonstrated to reduce significantly the incidence and reoccurrence of neural tube defects, such as spinia bifida of women (Gujska and Kuncewicz, 2005). The normal concentration of folic acid in human blood serum is 34.4 ± 10.4 nmol/L (Woo et al., 1999). Therefore, the sensitive determination of FA is very important for the clinical point of view. Numerous methods have been used for the quantification of FA including electrochemical (Wan and Yang, 2002; Wei et al., 2006; Vaze and Srivastava, 2007; Beitollahi et al., 2008; Wang et al., 2006a,b; Guo et al., 2006; Xiao et al., 2008; Lermo et al., 2009), spectrophotometry (Rao et al., 1978), flow injection chemiluminescence (Warthan, 1994), fluorimetric (Lapa et al., 1997) and high-performance liquid chromatography (Dong et al., 1988).

Among the different methods, electrochemical methods have received vast interest in recent years because they are less expensive, more convenient, more selective and sensitive. Han et al. have studied the electrochemical reduction and adsorption behavior of FA at mercury electrode using alternating current adsorptive stripping voltammetry and they found that FA was detectable at 2×10^{-12} mol l^{-1} through 10 min accumulation (Han et al., 1991). Blanco and coworkers have studied the adsorption behavior of FA using adsorptive stripping voltammetry and they reported the detection limit of 1.0×10^{-11} M at mercury electrode for the accumulation time of 10 min (Alvarez et al., 1987). Using cathodic stripping voltammetry, a detection limit of 9.0×10^{-11} M was reported for FA at hanging mercury drop electrode (Gall and Van den Berg, 1993). Although the detection limit of FA by the stripping voltammetry is very low, their application in the direct determination of FA is limited due to its poor reproducibility. To resolve this problem, chemically modified electrodes have been used for the quantification of FA including 2-mercaptobenzothiazole self-assembled monolayer (Wan and Yang, 2002) and multi-walled carbon nanotube modified gold electrodes (Wei et al., 2006), calixarene (Vaze and Srivastava, 2007)

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and palmitic and stearic acids (El Maali, 1992) modified carbon paste electrodes, 2,2′-[1,2-ethanediylbis(nitriloethylidyne)]-bis-hydroquinone double-wall carbon nanotube paste electrode (Beithollahi et al. 2008), single-wall carbon nanotube film (Wang et al., 2006a,b), lead film (Korolczuk and Tyszczuk, 2007), phosphomolybdic-polypyrrole film (Guo et al., 2006) and single-walled carbon nanotube-ionic liquid (Xiao et al., 2008) modified GC electrodes.

Although several reports have been published for the quantification of FA, no report has been published for the determination of FA in the presence of very important physiological interferents such as ascorbic acid (AA) and uric acid (UA). In the present study, we have determined FA in the presence of AA and UA at physiological pH using electropolymerized film of 5-amino-2-mercapto-1,3,4-thiadiazole modified GC electrode. The practical application of the present modified electrode was successfully demonstrated by the determination of FA in human blood serum samples.

2. Experimental

2.1. Chemicals

5-Amino-2-mercapto-1,3,4-thiadiazole (AMT), folic acid (FA), ascorbic acid (AA) and uric acid (UA) were purchased from Aldrich and were used as received. All other chemicals used in this investigation were of analytical grade. pH 7.2 phosphate buffer (PB) solution was prepared using Na_2HPO_4 and NaH_2PO_4. Double distilled water was used to prepare the solutions used in this investigation.

2.2. Instrumentation

Electrochemical measurements were performed in a conventional two compartment three electrode cell with a mirror polished 3 mm GC as the working electrode, Pt wire as counter electrode and a NaCl saturated Ag/AgCl as reference electrode. The electrochemical measurements were carried out with CHI Model 643B (Austin, TX, USA) electrochemical workstation.

In cyclic voltammetry, the electrochemical oxidations of AA, UA and FA were carried out at a scan rate 50 mV s^{-1}. Pulse width = 0.06 s, amplitude = 0.05 V, sample period = 0.02 s and pulse period = 0.20 s were used in differential pulse voltammetry (DPV). For chronoamperometric measurement, pulse width = 0.25 s and potential step = 1 were used. All the electrochemical measurements were carried out under nitrogen atmosphere at 27° C.

2.3. Fabrication of p-AMT modified GC electrode

Prior to modification, the GC electrode was polished with 0.50 and 0.05 μm alumina slurries and then rinsed thoroughly with water. Then, the electrode was sonicated in water for 5 minutes to remove adsorbed alumina on the electrode surface. Electropolymerization of AMT on the GC electrode was carried out by 15 successive potential sweeps between −0.20 and +1.70 V at a scan rate of 50 mV s^{-1} in 1 mM AMT containing 0.10 M H_2SO_4. By this procedure, an ultrathin film of p-AMT with a thickness of 25 nm was formed on GC electrode (Kalimuthu and John, 2009).

3. Results and discussion

3.1. Electrochemical behaviors of FA at bare and p-AMT film modified GC electrodes

We have examined the electrocatalytic activity of p-AMT film deposited on GC electrode by varying the potential cycles. We found

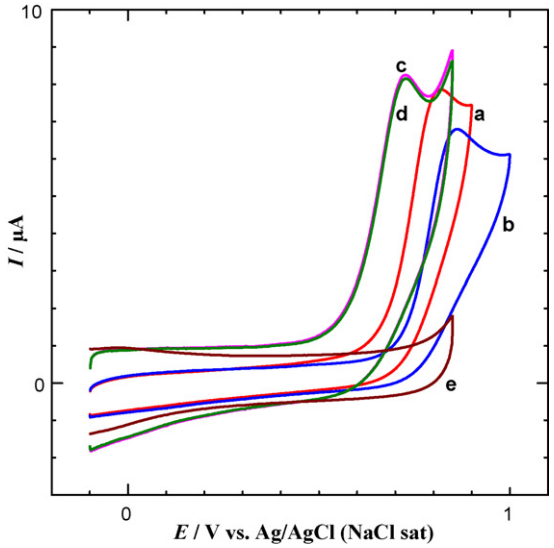

Fig. 1. CVs obtained for 0.5 mM FA at bare and p-AMT film modified GC electrodes after 1st (a and c) and 15 cycles (b and d) in 0.2 M PB solution at a scan rate of 50 mV s^{-1}. (e) CV obtained for p-AMT film modified electrode in the absence of 0.5 mM FA in 0.2 M PB solution.

that the polymer film deposited by 15 cycles showed higher electrocatalytic activity towards AA, UA and FA than the films deposited by more than 15 cycles. Thus, p-AMT film deposited by 15 cycles was chosen for all the electrochemical measurements. Fig. 1 shows the cyclic voltammograms (CVs) obtained for 0.50 mM FA at bare and p-AMT film modified GC electrodes in 0.20 M phosphate buffer (PB) solution (pH 7.2). At bare GC electrode, an oxidation peak was observed for FA at 0.82 V in the first cycle (curve a). In the subsequent cycles, the FA oxidation peak was shifted to more positive potential with decreased peak current. After 15 cycles, the oxidation peak of FA was observed at 0.86 V (curve b), indicating that bare GC electrode is not suitable for the stable determination of FA. The adsorption of oxidized product of FA on electrode surface is the possible reason for the decreased FA oxidation current and more positive shift in the oxidation potential at bare GC electrode. On the other hand, a well defined oxidation peak was observed at 0.72 V for FA at p-AMT film modified electrode (curve c), which is 140 mV less positive potential than at bare GC electrode. Unlike bare GC electrode, FA oxidation peak is highly stable at p-AMT film modified electrode in the subsequent cycles (curve d). This indicated that p-AMT film effectively prevents the fouling caused by the oxidized products of FA. The observed oxidation peak for FA in Fig. 1 is due to the two electron oxidation of FA to dehydrofolic acid (Dryhurst, 1977) as shown in Scheme S1 in the Supporting information. The p-AMT film does not show any redox peak in the absence of FA (curve e).

Further, to understand the fast electron transfer reaction of FA at p-AMT film modified electrode quantitatively, we have calculated the standard heterogeneous rate constant (k_s) for FA at p-AMT and bare GC electrodes using Velasco equation (Velasco, 1997) as given below.

$$k_s = 1.11\, D_o^{1/2} (E_p - E_{p/2})^{-1/2} \nu^{1/2}$$

where, k_s is standard heterogeneous rate constant; D_o is apparent diffusion coefficient; E_p is oxidation peak potential; $E_{p/2}$ is half-wave oxidation peak potential and ν is scan rate.

In order to determine the k_s it is necessary to find the diffusion coefficient for FA. The apparent diffusion coefficient (D_o) value was determined by a single potential chronoamperometry technique based on the Cottrell slope obtained by plotting current versus $1/\sqrt{}$time. Chronoamperometry measurements were carried out for