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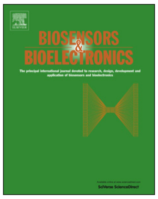

Development of α-polyoxometalate–polypyrrole–Au nanoparticles modified sensor applied for detection of folic acid

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ABSTRACT

In this work, electrochemically synthesized gold nanoparticles (AuNPs) and α-polyoxometalate (α-POM) ($K_7PMO_2W_9O_{39}\cdot H_2O$) were simultaneously doped into electropolymerized polypyrrole (PPy) film using the cyclic voltammetry (CV) technique. Scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and CVs were used to characterize the composite films. The PPy–α-POM–AuNPs modified gold (Au) electrode was used to determine folic acid (FA) using square-wave voltammetry (SWV). The modified electrode exhibited excellent electrocatalytic ability to the reduction of FA at 0.3 V (vs. SCE) with the electron transfer rate constant (k_s) of $1.15\times10^{-19}\ s^{-1}$. The common coexisting substances showed no interferences on the response of modified electrode to FA. The modified electrode indicated reproducible behavior and a high level stability during the experiments, making it particularly suitable for the analytical purposes.

1. Introduction

Vitamins are a group of organic compounds which are necessary in trace levels for the normal functioning and regulating the metabolic activity of the body (Ball, 2004; Lehninger, 1982). Folates, which belong to a group of water-soluble B9 vitamin compounds, are mandatory for the bio-synthesis of purines, pyrimidines and methionine. Folic acid or vitamin B9, known as (N-[p {[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino}benzoyl]-L-glutamic acid, is a water-soluble vitamin which acts as the co-enzyme in the regeneration of methionine from homocysteine (Pfeiffer et al., 2004). Moreover, FA with vitamin B12 participates in the nucleotide synthesis, gene expression and cell division. Deficiency of vitamin B9 results in anemia and may increase the possibility of heart attack (Hoegger et al., 2007). The deficiency of vitamins that induces different types of abnormalities may be corrected by administering the vitamins from natural and/or artificial sources. The most effective technological approaches to combat deficiency typically FA in developing countries include supplementation targeted at high-risk groups combined with a program of food fortification and dietary strategies designed to maximize the bio-availability of both the added and the intrinsic food vitamins. There is, thus, a large and rapidly growing interest on developing the analytical methods which could determine contents at sub-micromolar levels in different matrices, especially in food stuffs and supplements. Common analytical methods for the determination of FA (as a single species) in real samples are LC/MS/MS (Nelson et al., 2006), HPLC (Quiros et al., 2004), capillary electrophoresis (CE) (Zhaoa et al., 2006) and chemiluminescence methods (Zhang et al., 2008). The uses of these analytical methods are often complicated, time consuming, expensive, need professional operators and require multistage steps. Besides, they cannot be used by clinical laboratories for rutting analysis. Thus, simple methods are expected and attention has been paid on them. Electrochemical methods are desirable for the determinations, compared to other technologies due to low cost, simple procedure and high sensitivity. To fabricate suitable modified electrodes for analysis of electroactive substances, introductions of new synthetic substances, modifiers and procedures are considered as a challenge for analytical chemists. Therefore, electroactive polymers have been an area of immense interest over the past 30 years, since the first discovery of conducting poly-acetylene in 1977 (Shirakawa et al., 1977). Extensive research on several conjugated polymers, including poly(p-phenylene), polyaniline(PANI), PPy, polythiophene, polyindole, polycarbazole, polyfluorene, poly(p-phenylene vinylene), and their substituted derivatives, has led to their applications in rechargeable batteries, microelectronics, sensors, electrochromic displays, and light-emitting and photovoltaic devices (Caruso et al., 1998). Therefore,

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conjugated polymers such as PANIs and PPys have received special recognition in electrochemistry owing to their good stability and interesting redox behavior (Guo et al., 2006; Kurth et al., 2000). Polyoxometalates are anionic structures constituted of rare transition-metal elements in their highest oxidation state and usually as cluster structures. They are remarkable in several respects, including the multitude of their properties based on their sizes, shapes, charge densities and reversible redox potentials or their enormous diversity of structures (Hoegger et al., 2007; Hu et al., 1999; Liu et al., 1999; Muller et al., 1997; Wang et al., 1997). Seldom works have been reported on use of different types of POMs to construct film modified electrodes (Caruso et al., 1998; Ichinose et al., 1998; Kurth et al., 2000, Cheng and Otero, 2002; Guo et al., 2006). Synthesis of new POMs and fabrication of POMs based electrodes are yet in demand and developments. Guo et al. (2006) reported a POM based sensitive electrode to FA at more negative reduction potential and determinination based on the high inhibitory activity toward the reduction of modified electrode in acidic pHs. Therefore, we here intended to construct a PPy modified electrode based on the synthetic α-POM and its incorporation with AuNPs. The incorporation of conductive properties of PPy film, the electron pathway effect of α-POM and good capabilities of AuNPs in electrochemical sensing (i.e. high surface to volume and electrocatalytic effect) was done in situ in the PPy–α-POM–AuNPs modified gold electrode for sensitive monitoring of FA at more positive reduction potentials which have not been obtained with the other sensors as in Table S1. In the proposed PPy–α-POM–AuNPs modified electrode, easier conditions have been obtained and FA is determined directly. Therefore, the superior performances of the modified electrode permit its recommendation for determining FA in biological and non-biological samples with confidence at biological pHs.

2. Experimental

2.1. Reagents and chemicals

Folic acid, sugars, vitamins, amino acids, $HAuCl_4 \cdot 3H_2O$, nitrate salts of metal ions, and other chemicals were of analytical reagent grade from Merck or Fluka Company. Double distilled deionized water was used for preparing all solutions throughout the experiments. Stock solutions of FA were freshly prepared as required and protected from light during the investigation. Britton–Robinson (B–R) buffer solutions were prepared by adding appropriate amounts of 0.5 M of sodium hydroxide solution into solutions containing a mixture of 0.1 mol L^{-1} of boric, acetic and phosphoric acids to produce the pHs over 4.0–12.0. The α-POM was also synthesized in accordance with the pervious procedure reported (Rafiee et al., 2009), and it was used successfully to fabricate the proposed sensor.

2.2. Apparatus

Electrochemical experiments were carried out using a 797 VA computerized (Metrohm). A conventional three electrodes system was employed incorporating a working modified electrode as PPy–α-POM–AuNPs, a saturated Ag/AgCl reference electrode and a Pt wire counter-electrode. All potentials were reported vs. the Ag/AgCl reference electrode. The pH measurements were performed using a 781 pH/ion meter equipped with a combined glass electrode. The surface morphology of modified electrodes was characterized with a scanning electron microscope (SEM) (Philips XL 30) with gold coating.

2.3. Electrode modification

An overall schematic of thin film formation for PPy–α-POM doped with in situ synthesized AuNPs is shown in Fig. 1A. To prepare a modified electrode, the Au bare electrode was polished with emery paper followed by alumina (1.0 and 0.05 μm) and then thoroughly washed with double-distilled water. Then, the electrode was placed in an ethanol and water container and bath ultrasonic cleaner was used to remove adsorbed particles. The resulting Au bare electrode was then immersed in B–R buffer solution (pH 6) containing 50 mg of α-POM salt, 20 mg of pyrrole monomer and 10 mg of $HAuCl_4 \cdot 3H_2O$ in 25 ml completion volume and the electrode potential was cycled between −1.0 and 1.0 V at a scan rate of 50 mV s^{-1} for 20 cycles. During the polymerization process of pyrrole, grafting of α-POM was done. The Au(III) ions were reduced to AuNPs, simultaneously. The effect of scan number (N) on the construction of the composite film was investigated. Through changing the scan number, the amount and thickness of the composite film could be controlled. The peak currents of FA enhanced steadily with increasing of scan number up to 20 scans, indicating the continuous deposition of recognition element (PPy–α-POM–AuNPs film) at the electrode surface. For $N > 20$, the peak currents of FA decreased gradually; this may be due to increase in the electrical resistance of the polymer film. In the absence of AuNPs and α-POM in PPy–α-POM and PPy–AuNPs constructed films, the optimum scan numbers were obtained to be $N < 20$ with lower FA peak currents (this is due to high electron transfer resistance of films in the absence of AuNPs and α-POM).

2.4. Supplement tablet preparation

Vitamin supplement formulations containing FA and other components were analyzed with the proposed modified electrode. Twenty tablets of each sample were weighed to calculate the average tablet weight. They were finely powdered and homogenized. A quantity of the resulting powder equivalent to the weighted average of one tablet was accurately weighed and transferred into a glass vessel; 25 ml of distilled de-ionized water was also added and magnetically stirred for 10 min. The resultant mixture was filtered, and its ionic strength was adjusted to 0.5 mol L^{-1} with $NaNO_3$ and the pH to 6.0 ± 0.1 with the Briton Robinson buffer solutions before volume completion. The resultant solution was quantitatively transferred into a volumetric flask and diluted to 100 ml using distilled de-ionized water, keeping the pH at 6 ± 0.1. Then, an aliquot of 10 ml resulting solution was analyzed using the proposed modified electrode using a standard addition method.

2.5. Human serum sample preparation

Taking into account ethic and health considerations, human serum samples were collected from the volunteers by a pathology laboratory in Kermanshah city. Into a centrifuge tube (10 ml), an aliquot (1 ml) of the human serum sample was transferred and diluted with ethanol as a serum protein precipitator. After overtaxing for 30 s, the precipitated protein was separated out by centrifugation for 3 min at 12,000 rpm. The clear supernatant layer was filtered through a 0.45-μm mille-pore filter to produce a protein-free human serum. The resulting serum sample was then analyzed by the proposed sensor for the vitamin B9 detection, using the spiked amounts and the standard addition method at pH 6.0.

3. Results and discussion

3.1. Electrochemical characterization of the PPy–α-POM–AuNPs Au electrode

Electropolymerization is widely accepted as an appropriate methodology for the preparation of suitable nanomaterials. This is because, the polymer characteristics such as film thickness, permeation and charge transport properties can be easily controlled