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A new droplet-based polymeric banana electrochemical biosensor for analysis of one microliter solution of paracetamol

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ABSTRACT

Modern researches and also the laboratories increasingly need the methods to measure the small volumes as microliter of analytical samples for determination of various analytes with a high accuracy. An electrochemical biosensor based on a modified carbon paste electrode with hydrogel as an absorbent polymeric matrix and banana as a source of polyphenol oxidase was constructed and it was used for determination the concentration of paracetamol in one microliter volume of solution. The electrochemical oxidation of paracetamol was studied by cyclic voltammetry and square wave voltammetry. The effective parameters on the voltammetric response of the constructed biosensor were optimized. The results obtained by cyclic voltammetry revealed that the banana-hydrogel carbon paste electrode shows a higher current response compared to a banana carbon paste electrode and hydrogel carbon paste electrode. Because of high absorption property of hydrogel, the proposed electrode has the capability to measure only one μL of sample solution, which is very important and considerable property for limited volumes of sample solutions. The modified electrode also exhibits a low detection limit (1.6 μM) with a good linear range (10–250 μM) and it has a good reproducibility and stability in both basic and acidic environments even in the presence of the enzyme and a long life time (20 days) for measurement of paracetamol in solutions.

1. Introduction

Plant tissues have received considerable interest in biosensor construction. For construction an enzyme biosensor, two sources of enzyme: the pure enzyme and plant tissue can be employed. Plant tissue biosensors use thin slices of plant as a source of the enzyme. The plant tissue has low cost, high stability, long life time but poor selectivity and a long response time. The diversity and availability of plants, no need sample preparation steps, the presence of necessary cofactors, are other advantages of the plant tissue for construction of biosensors [1–9].

The procedures most commonly used for the construction of the electrochemical biosensors, are the retention of a thin slice of plant, directly fixed on the surface of the electrode and mixing the tissue with a carbon paste matrix [3]. Other methods, are the immobilization of plant tissue in alginate and hydrogels [10].

The hydrogels are hydrophile, porous, insoluble, cross-linked polymers, containing ionic functional groups. These polymers can absorb and retain fluids, such as water, biological fluids such as blood and urine. Hydrogels are also smart materials that respond to some stimuli such as pH, temperature and light. The pH responsive hydrogels have acidic or basic side groups that are ionizable and their charge will be a function of the pH value of the solutions. The main advantages of these hydrogels in the biosensor field, are biocompatibility and their ability to be responsive to physiological stimuli [10–17].

Banana, potato, apple, avocado, coconut, mushroom and other species of plants have the tyrosinase or polyphenol oxidase (PPO) enzyme. PPO catalyses two different reactions, the o-hydroxylation of monophenols to o-diphenols and the subsequent oxidation of the o-diphenols to o-quinones [18,19].

Acetaminophenol or paracetamol (PA), is a common analgesic and antipyretic drug that is used for the alleviation of fever, backaches, headaches and pains. Because of the exigent roles of paracetamol, its determination is important in drugs and biological fluids [20–28]. Many analytical methodologies such as optical methods [29,30], chromatographic methods [31–34], electroanalytical methods [35,36] and capillary electrophoretic methods [37,38] have been proposed for the determination of paracetamol in solutions.

Low sample volume consumption is an important aspect of technical analysis. For samples that are restricted in volume, we need

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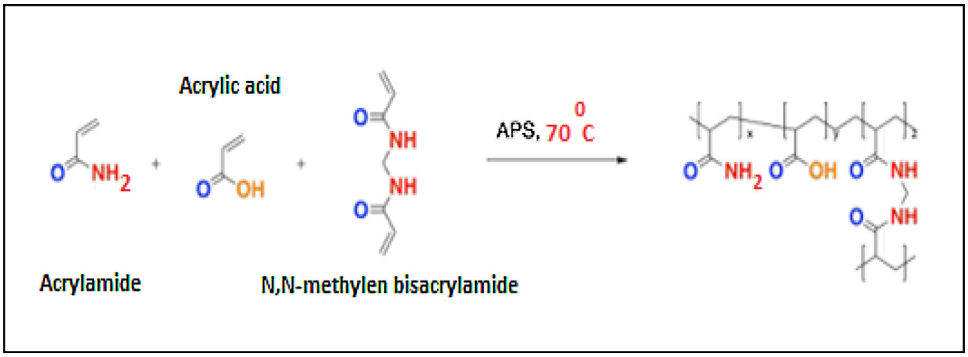

Fig. 1. Chemical structure of monomers (Acrylamide, Acrylic acid), crosslinker (*N,N*-methylen bisacrylamide) and reaction path for hydrogel synthesis.

the methods that consume a low volume of sample solution for detection process.

In this paper, we describe the determination of paracetamol with a new droplet based banana- hydrogel carbon paste electrode. It offers interesting advantages, such as: good selectivity because of the presence of the enzyme, rapidity, good stability in both acidic and basic media, ease of preparation, low cost and also it needs a very low volume of sample solution. The method for fabrication of the electrochemical biosensor and its characterization are also reported.

2. Material and methods

2.1. Apparatus

All experiments were carried out in a 15 ml glass cell at 25 °C. The voltammetric measurements were performed using a μAutolab electrochemical system (Metrohm) equipped with NOVA software. The electrochemical cell was assembled with a three electrode system; an Ag/AgCl reference electrode, a platinum counter electrode (both from Azar electrode Co, Urmia Iran) and a constructed banana-hydrogel carbon paste electrode (BH-CPE) as a working electrode.

2.2. Reagents and solutions

All chemical reagents were of analytical-reagent grade and dionized distilled water was used for preparation of the solutions. Acrylamide (AM) and acrylic acid (AA) monomers, ammonium persulfate (APS), *N,N*'-methylenebisacrylamide (MBA), sodium hydroxide and hydrochloric acid (37%w/w) were purchased from Merck chemical company. Chitosan, molecular weight: 100,000–300,000 was purchased from Across organics. Paracetamol (PA) was purchased from Sigma and a 2.5×10^{-2} mol l^{-1} stock solution of this drug in 0.10 mol l^{-1} phosphate buffer solution was prepared. For preparation of the standard solutions, the stock solution was diluted with adequate phosphate buffer solution.

2.3. Preparation of the hydrogel

The hydrogel [39–41] used in this work, was synthesized according to the following procedure [39]: 150 ml solution of acrylamide (AM) and neutralized acrylic acid (AA) monomers (AA/AM mol ratio: 0.8) with total concentration of 5% wt, was prepared in dionized distilled water in a volumetric flask. Before preparation of the solution, the AA monomer was neutralized at 5 °C with sodium hydroxide solution (2 M). Then, 8.8 mg of *N,N*'-methylenebisacrylamide (MBA) as a cross linker was added to the above monomers solution. After purging the solution with N_2 for 30 min, the solution was heated at 50 °C and 35.2 mg of ammonium persulfate (APS) as an initiator was added into the flask. The solution was stirred vigorously for 3 h to complete the polymerization process. The prepared hydrogel was washed with water and ethanol respectively, and then dried in an oven at 70 °C. Finally, the dried sample was milled with a mortar and it was used for further characterization. Fig. 1, shows the chemical structures of acrylamide, acrylic acid, *N,N*'-methylenebisacrylamide and the synthesized hydrogel [42]. A typical FT-IR pattern of the hydrogel (Fig. 2) confirmed the presence of the functional groups in synthesized hydrogel.

2.4. Construction of the electrochemical biosensors

0.1 g of the banana tissue was ground in a mortar and mixed with 0.2125 g of graphite powder and 0.0375 g of hydrogel, and then 0.05 g of paraffin oil was added and mixed thoroughly for preparation of banana-hydrogel carbon paste electrode (BH-CPE). Also a hydrogel carbon paste electrode (H-CPE) without banana tissue and banana carbon paste electrode (B-CPE) without hydrogel, were prepared for investigation of the performance of the modified electrode. A portion of each paste was packed into the tip of a 1 ml insulin plastic syringe and a copper wire was inserted to obtain the electrical contact. Prior to use, the surface of the electrode was polished with a weighing paper.

3. Results and discussion

3.1. Electrode characterization

The scanning electron microscopy (SEM) is a type of electron microscopy that routinely used to generate high-resolution images of the surface of the solid samples. The surface morphologies of the banana-hydrogel modified electrode were investigated using SEM. As is evident in Fig. 3, the surface topography for the banana-hydrogel electrode shows a homogeneous surface.

3.2. Principle of the electrochemical measurements

The polyphenol oxidase at the electrode surface, catalyses the oxidation of paracetamol (PA) to *n*-acetyl-*p*-benzoquinoneimine (NAPQI) and this product is electrochemically reduced to paracetamol [6]. The oxidation of PA, is a two-electron transfer process (Fig. 4). All measurements were carried out in a 15 ml glass cell in 5 ml of phosphate buffer solution (0.1 mol l^{-1}). The cyclic voltammograms were obtained by cycling the potential between −0.35 and +1 V at scan rate of 100 mV s^{-1}. The square-wave voltammetric (SWV) measurements were performed at the potentials between −0.1 and +1 V.