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Amperometric uric acid biosensor based on poly(vinylferrocene)-gelatin-carboxylated multiwalled carbon nanotube modified glassy carbon electrode

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ABSTRACT

In this study, a new uric acid biosensor was constructed based on ferrocene containing polymer poly (vinylferrocene) (PVF), carboxylated multiwalled carbon nanotubes (c-MWCNT) and gelatin (GEL) modified glassy carbon electrode (GCE). Uricase enzyme (UOx) was immobilized covalently through *N*-ethyl-*N*′-(3-dimethyaminopropyl) carbodiimide (EDC) and *N*-hydroxyl succinimide (NHS) chemistry onto c-MWCNT/GEL/PVF/GCE. The c-MWCNT/GEL/PVF composite was characterized by scanning electron microscopy, cyclic voltammetry and electrochemical impedance spectroscopy. Various experimental parameters such as pH, applied potential, enzyme loading, PVF and c-MWCNT concentration were investigated in detail. Under the optimal conditions the dynamic linear range of uric acid was 2.0×10^{-7} M – 7.1×10^{-4} M (R=0.9993) with the detection limit low to 2.3×10^{-8} M. With good selectivity and sensitivity, the biosensor was successfully applied to determine the uric acid in human serum. The results of the biosensor were in good agreement with those obtained from standard method. Therefore, the presented biosensor could be a good promise for practical applications in real samples.

1. Introduction

The rapid, accurate, reliable and inexpensive detection of uric acid in human biological fluids is of great importance in the diagnosis and treatment of several disorders such as gout [1], renal disease [2] and Lesch-Nyhan syndrome [3]. The normal level of uric acid in serum is between 240 and 520 μM [4]. Elevated levels of uric acid in serum are known as hyperuricemia and hyperuricemia has been found to be associated with hypertension [5], metabolic syndrome [6] and cardiovascular disease [7]. Many techniques such as, fluorescence [8], spectrophotometry [9], HPLC-mass spectrometry [10], ion chromatography [11], colorimetry [12], chemiluminescence [13], electrochemistry [14] and electrochemical biosensors [15] have been reported for uric acid detection. Among these techniques, amperometric biosensors provide advantages such as high selectivity and sensitivity, direct measurement, low cost and rapid response [16].

Carbon nanotubes (CNTs) are interesting type of the carbon derivatives offering unique geometrical, mechanical, electronic and chemical properties. CNTs have been extensively researched for sensing applications including fabrication of biosensors because of excellent electrical properties, high surface-to-volume ratio, and high chemical stability. Moreover, CNTs can be used for promoting electron-transfer between the electroactive species and electrode [17]. However, the major challenge for the preparation of CNTs-modified electrodes is the homogeneous dispersion of CNTs since the CNTs form large bundles due to strong van der Waals interactions [18]. Several methods have been investigated to get homogenous dispersion of CNTs [18,19]. Gelatin is a protein obtained from collogens by partial hydrolysis. Because of its many merits, such as its biological origin, biodegradability, nontoxicity, biocompatibility, film forming ability and commercial availability at low cost gelatin has been widely used in food and pharmaceuticals industry [20]. Gelatin also serves as a matrix for the assembly of biomolecules, nanoparticles and other substances [21]. Gelatin was used as an dispersive agent to obtain stable MWCNT dispersions [22,23]. Zheng and Zheng reported that the CNTs-gelatin dispersion was found to be stable for at least two weeks. This good stability was attributed to the immobilization of the non-polar amino acid chain of the gelatin in the side wall of CNTs through hydrophobic–hydrophobic interactions [23]. In this study, gelatin is selected to suspense CNTs owing to its favourable properties mentioned above.

Poly(vinylferrocene) is a conducting redox polymer that contains localized sites that may be oxidized and reduced. PVF is

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widely used as a fundamental system for modeling modified electrode polymer/electrolyte interfaces due to its simple electrochemistry (a reversible one electron process), high stability and the ease of coating of thin film using a variety of methods [24]. PVF was reported as a redox mediator for the oxidation of enzymatically produced hydrogen peroxide [25]. The use of PVF as an immobilization matrix for enzymes such as cholesterol oxidase, creatinase, sarcosine oxidase and urease has been reported in biosensor applications [25–27]. In these biosensors the oxidized form of the polymer (PVF^+) was used to bind/immobilize negatively charged enzymes electrostatically above the isoelectric point of the enzymes. The use of PVF as a redox mediator in carbon paste electrodes was also reported [28,29]. In our previous work [26] we have immobilized creatinase and sarcosine oxidase electrostatically onto PVF^+ coated Pt electrode to construct an amperometric creatine biosensor. However, the storage stability of this biosensor was not satisfactory due to the weak interaction between the polymer matrix and enzymes. In order to achieve an increased lifetime stability of enzyme electrode, covalent linking of the enzymes on transducer is an efficient method of immobilization [30].

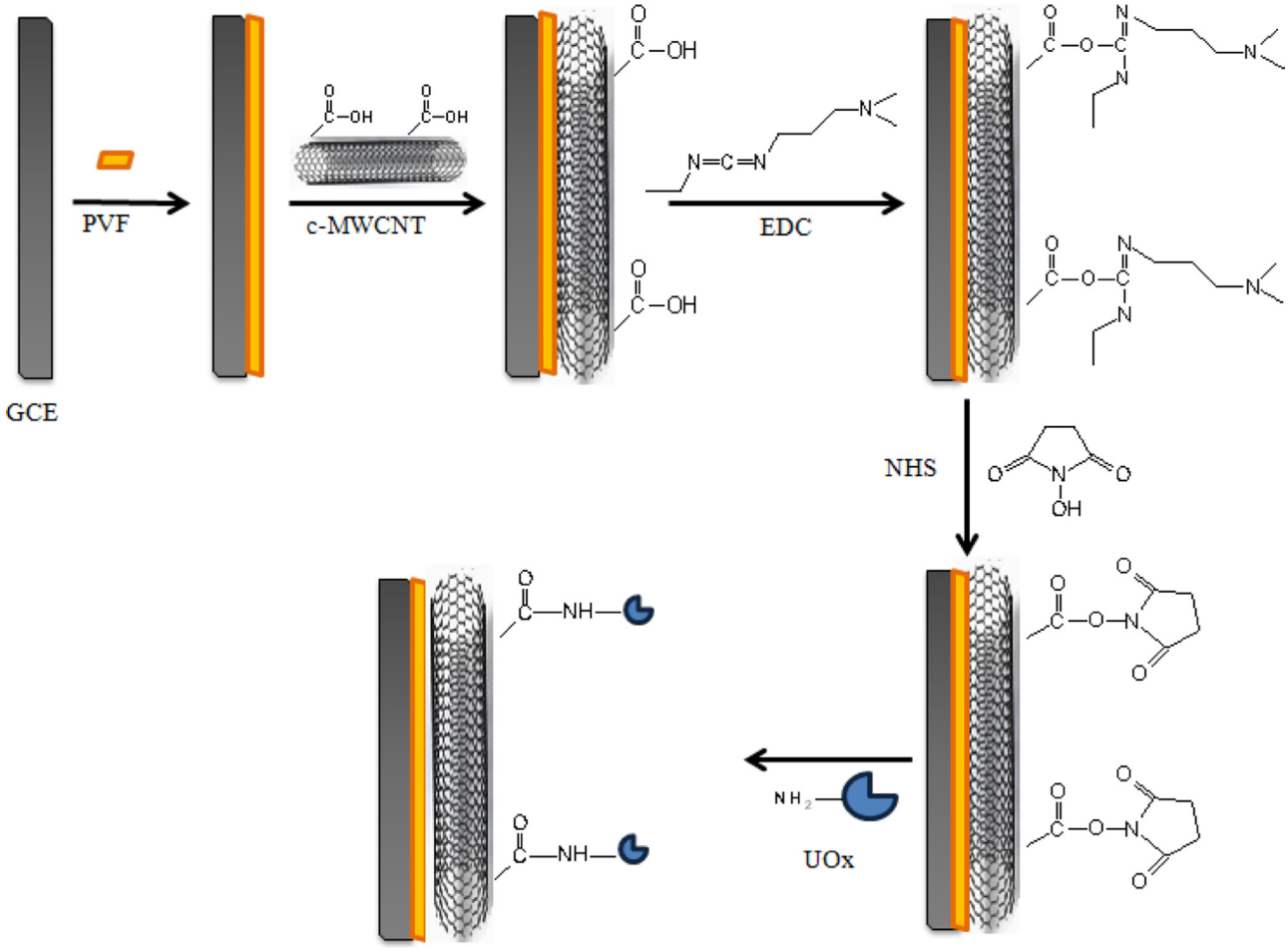
This article describes the fabrication of the uric acid biosensor based on carboxylated multiwalled carbon nanotubes, redox polymer PVF and covalently linked uricase. In previous articles, $[Fe(CN)_6]^{3-}$ [31], 5-methylphenazinium (MP) and 1-methoxy-5-methylphenazinium (MMP) [32] were reported to work as an electron acceptor for UOx in place of O_2. In this article, ferrocene containing polymer PVF was used as an useful electron acceptor for UOx. The experimental conditions and the performance parameters of the uric acid biosensor were studied. The successful application of the purposed biosensor for uric acid biosensing in real samples was also described.

2. Experimental

2.1. Equipment and reagents

The electrochemical studies were carried out using IVIUM electrochemical analyzer (Ivium Technologies, Netherlands) connected to a three-electrode cell stand (Bioanalytical Systems, Inc., USA). The working electrode was a modified glassy carbon electrode (BAS MF 2012). The counter and the reference electrodes were a Pt wire (BAS MW 1034) and Ag/AgCl electrode (BAS MF 2052) (Bioanalytical Systems, Inc., USA), respectively. Scanning electron microscopic (SEM) images were recorded on Carl Zeiss AG, EVO® 50 Series. The pH values of the buffer solutions were measured with ORION Model 720 A pH/ion meter and ORION combined pH electrode (Thermo Scientific, USA).

Uricase (E.C.3.5.3.3. from *Arthrobacter globiformis* sp. with a specific activity of 18 Units/mg solid), uric acid, *N*-ethyl-*N*′-(3-dimethyaminopropyl) carbodiimide, *N*-hydroxyl succinimide, gelatin (type A, porcine skin, analytical grade, G-2500), potassium hexacyanoferrate (III), potassium hexacyanoferrate (II) trihydrate, vinylferrocene and ascorbic acid were supplied from Sigma – Aldrich. Sodium monohydrogenphosphate, sodium dihydrogenphosphate and glucose were from Fluka. Carboxylated multiwalled carbon nanotubes (outer diameter <8 nm and length $10-30$ μm) were from Cheaptubes Inc. (Brattleboro, USA). All other chemicals were obtained from Merck. All aqueous solutions



Scheme 1. The stepwise fabrication process of the biosensor .