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Full Length Article

Non-enzymatic sensing of kidney dysfunction biomarker using pectin – MWCNT nanocomposite

K. Yazhini, S.K. Suja*, G. Jayanthi Kalaivani, J. Bagyalaksmi, S. Pavalamalar

Department of Chemistry, Lady Doak College, Madurai, India

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ABSTRACT

Creatinine, a biomarker for kidney dysfunction, is a molecule created within skeletal muscles as a product of physical activities. The normal physiological concentration of creatinine is 40–150 μM, beyond which it will lead to kidney dysfunction. Thus monitoring of creatinine level becomes more important for normal functioning of the body. In the currently available enzymatic studies, three enzymes were required for sensing creatinine. As the enzymatic electrochemical sensing studies are tedious and have several drawbacks, non-enzymatic sensing of creatinine using a biopolymer based sensor was proposed in the present study. Pectin (PEC), a naturally occurring polysaccharide, was extracted from musk melon peels and it was used for the modification of MWCNT. The prepared bionanocomposite was characterized using UV–vis, FT-IR, XRD, RAMAN, SEM, and TGA techniques. The bionanocomposite was effectively incorporated into the carbon paste matrix (CPE) and fabricated as a working electrode (CPE/PEC-MWCNT). The enhanced surface properties like electro active surface area A (0.0329 cm^2), surface concentration Γ (0.0503×10^{-6} mol cm^{-2}), diffusion coefficient D_0 (0.0964 cm^2 s^{-1}), heterogeneous rate constant k_s (0.3736 s^{-1}) of CPE/PEC-MWCNT as compared to that of GCE (Glassy Carbon electrode), CPE, CPE/PEC and CPE/MWCNT electrodes, suggested enhanced electrochemical functionalization, competent diffusion of creatinine and hence effective electron transfer at the newly fabricated electrode surface. The electrode was also effectively utilized for the sensing of creatinine in urine samples.

1. Introduction

Chronic end-stage kidney failure affects many patients in the world. The consequence of this disease is the significant increase in the level of metabolic waste products in blood including urea and creatinine. Creatinine (2-amino-1-methyl-5H-imidazol-4-one), the end product of the metabolism of creatine in mammals, is a clinically important kidney dysfunction biomarker. The amount of creatinine in urine is directly related to the amount of creatine and creatine phosphate present in the body. The normal concentration of creatinine ranges from 40 to 300 mg dl^{-1} (~3.6–27 mM) in male and 37–250 mg dl^{-1} (~3.3–22.5 mM) in female. Hence quantification of creatinine in various biological fluids is significant in clinical diagnostics for the evaluation of renal dysfunction, thyroid malfunction and muscle damage. Although, several highly selective, relatively fast detection and quantification methods like high-performance liquid chromatography (HPLC), colorimetry, enzymatic, amperometric methods etc., are available, these methods suffer from serious drawbacks. HPLC method requires several pre-preparations, expensive equipment etc., The amperometric creatinine biosensors are based on a multienzyme sequence proposed by Tsuchida and Yoda [1] consisting of three enzymes viz., creatinine amidohydrolase (CA, 1EC 3.5.2.10), creatine amidinohydrolase (CI, EC 3.5.3.3), and sarcosine oxidase (SO, EC 1.5.3.1). The performance of the biosensor depends on the thickness and the stability of the enzyme layer. Hence stabilization and calibration of the sensor is required and is not cost effective also. The current laboratory method relies on the determination of creatinine concentration using colorimetric determination of Jaffe reaction [2,3]. This method is simple, but suffers from the disadvantages of non- specificity and non- selectivity (in presence of interferences) [4,5]. Therefore the development of a cost effective, rapid, sensitive, selective and stable sensor is required for pertinent applications [6–9]. For the construction of a successful biomarker sensor, there should be minimum pretreatment of samples. The response should be accurate, precise, reproducible and linear over the concentration range of interest, without dilution or concentration. It should also be free from electrical or other transducer induced noise. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or

* Corresponding author.
E-mail address: suja@ldc.edu.in (S. S.K.).

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antigenic effects. The biosensor can provide real time analysis for rapid measurements of analytes from human samples. The complete biosensor should be cheap, small, portable and capable of being used by semi- skilled operators [10,11].

Hence the recent researches are growing in the area of non-enzymatic sensing of creatinine [12]. At present exploding research in the utilization of carbon based materials like graphene, carbon nanotubes, etc., as non-enzymatic sensing material are being carried out. At present several electrochemical sensing studies have been carried out using biopolymers like chitosan [13] as well as CNTs [14–16]. The research on CNT based carbohydrate biopolymer nanocomposite has also been progressing rapidly over the past few years. Such nanocomposites possess the exhilarating features of both CNTs and the carbohydrate biopolymers that they get utilized in wide variety of applications especially electrochemical sensing applications.

In the present study an attempt is made to synthesize a new kind of bionanocomposite by suitable modification of MWCNT with pectin (PEC) (a carbohydrate polymer). The newly prepared bionanocomposite was used for the non-enzymatic electrochemical sensing of the kidney dysfunction biomarker, creatinine. Among the various electrochemical techniques, cyclic voltammetry (CV) and Differential Pulse Voltammetry (DPV), three electrode potentiostats were chosen for the study as they are simple, reliable and sensitive [17]. For the working electrode, several materials such as mercury, platinum, gold, silver, carbon etc., are used normally. Among other electrodes, carbon paste electrodes (CPEs) have advantages like ease of preparation, chemical inertness, robustness, renewability, stable response, low ohmic resistance, no need for internal solution and suitability for variety of sensing and detection applications.

In the present work, the Carbon Paste Electrode (CPE) is modified using the biopolymer, pectin and Multi Walled Carbon NanoTube (MWCNT). Pectin can be extracted from suitable agro-by-products like citrus peel and apple pomace and used in the food industry as natural ingredients for their gelling, thickening, and stabilizing properties [18]. In the present study, pectin was extracted from musk melon peels, which can be considered as an effective way of minimizing solid waste. Hence the CPE modified with PEC and MWCNT, CPE/PEC-MWCNT can be effectively utilized for non enzymatic creatinine sensing.

2. Experimental

2.1. Chemicals and materials

Paraffin liquid, Potassium ferricyanide, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate were obtained from Merck and used as received without further purification. Graphite powder was obtained from Loba Chemie, Nitric acid from Emplura and (MWCNT) from Sisco Research Laboratories. All the solutions were prepared in millipore water. Standard laboratory procedures were followed for the preparation of solutions. Appropriate quantities of Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate were mixed to prepare phosphate buffer solutions of varying pH. The stock solutions of dextrose (0.1 M), urea (0.1 M), ascorbic acid (1 mM) and uric acid (1 mM) were prepared by using millipore water.

2.2. Instrumentation

UV–vis absorption spectra were recorded using JASCO UV–vis 360 spectrophotometer. The Fourier Transform Infra Red (FT-IR) spectra of the samples in KBr pellets were recorded on a FTIR-8400S SHIMADZU spectrometer. X-Ray diffraction (XRD) patterns

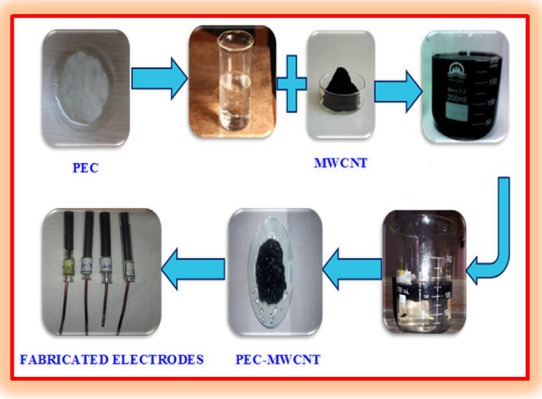

Fig. 1. Synthesis of PEC-MWCNT composite and Fabrication of Electrodes.

of PEC, MWCNT and composite were carried out using X'Pert PRO family of multipurpose Analytical X-ray diffractometer. The surface morphology of the pectin, MWCNT and the composite was observed by using Scanning Electron Microscope (SEM) – SIGMA ZEISS. The Thermo Gravimetric Analysis (TGA) was recorded on a NETZSCH STA Jupiter instrument. The Raman spectra of a sample were recorded on Imaging Spectrograph STR 500 nm focal length laser Raman Spectrometer, SEKI, Japan. The cyclic voltammetric measurements were performed on a CHI6063C Electrochemical analyzer. A three electrode system including Ag/AgCl (saturated KCl) as reference electrode, platinum wire as counter electrode and modified CPE as working electrode was employed for both cyclic voltammetric and Differential Pulse voltammetric studies. The electrolyte solutions were purged with N_2 for about 5 min to remove O_2 and kept under N_2 atmosphere throughout the measurements.

2.3. Preparation of pectin

PEC was extracted from the peels of musk melon. 100 g of fresh peels of musk melon were weighed and thoroughly washed with water. 0.5N HNO_3 was added to it and heated for 20 min. The resulting solution was stirred well and filtered using Bucher funnel. To the filtrate equal amount of 95% ethanol was added. The PEC was formed and it was then filtered using Buchner funnel.

2.4. Synthesis of PEC-MWCNT composite

The PEC-MWCNT composite was synthesized using solvent casting method. 3 g pectin was dissolved in 50 ml of Millipore water. About 0.3 g of MWCNT was added and dispersed. It was ultrasonicated for about 3–5 h. The mixture was then poured into equal amount of ethanol taken in a beaker. The composite so formed raised to the surface, which can be filtered using Buchner funnel and was dried. The prepared PEC-MWCNT bionanocomposite was then used for the preparation of working electrodes. The schematic representation of synthesis of the composite was shown in Fig. 1.

2.5. Fabrication of the electrode

A homogeneous carbon paste was prepared by hand mixing 700 mg of graphite powder and 3 drops (~300 mg) of paraffin oil in an agate mortar. The bare CPE was then prepared by packing this paste into a 4 cm length hollow glass tube of 3 mm diameter and the surface was smoothened on a filter paper. The electrical contact was provided by a copper wire. For the preparation of CPE/PEC-MWCNT electrode, the hollow glass tube was filled by mixing 350 mg composite and 350 mg graphite powder in an agate mortar with 3 drops (~300 mg) of paraffin oil. The sensitivity of CPE/PEC-MWCNT elec-

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