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Full Length Article

Fabrication of a molecularly imprinted silylated graphene oxide polymer for sensing and quantification of creatinine in blood and urine samples

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ABSTRACT

A graphene oxide (GO)-based electrochemical sensor, trimethyl silane propyl methacrylate-GO copolymerized with 2-hydroxymethacrylate/methyl methacrylate [TMSPMA-GO-co-HEMA/MMA], to detect creatinine (Cn) was fabricated by molecular imprinting technology followed by electrochemical detection. Electrochemical measurements were made by cyclic voltammetry and differential pulse voltammetry. Polymerization of GO-TMSPMA was achieved in the presence of Cn with ethylene glycol dimethacrylate as crosslinker and 2,2′-azobisisobutyronitrile as initiator. The physical and chemical properties of the resulted material were characterized by FTIR spectroscopy, NMR spectroscopy, Raman spectroscopy, XRD, SEM, and AFM. The effective pH for the response of the sensor material was found to be 7.4. The electrode exhibited a response time of ~2 min, and the limit of detection obtained was 0.1878 mg/dL and the limit of quantification was 0.6122 mg/dL. The fabricated molecular imprinted polymer sensor could be reused several times without decrease in its selectivity. The feasibility of the present sensor in real time was successfully verified by analyzing Cn level in human blood serum and urine samples. By comparing the present sensing method with the traditional Jaffe method, we obtained a linear correlation with a coefficient of 0.9928; this implies that the present biosensor possesses a sensitivity comparable to that of the traditional laboratory method.

1. Introduction

Creatinine (Cn) is a breakdown product of creatine, which is an important compound in muscles, and is normally removed from the blood by the kidneys [1]. An increase in Cn level in the blood means that the kidneys are not functioning well; as the kidney function slows down, the Cn level increases [2]. Kidney dysfunctions can be estimated by checking the Cn level in the blood and urine. In contrast to urea, Cn concentration in body fluids is not influenced by protein intake. Cn level is a more reliable indicator of renal dysfunction. It is important to maintain a normal level of Cn because a low level may be indicative of muscular dystrophy and myasthenia, while a high level is indicative of diabetic nephropathy, glomerulonephritis, pyelonephritis, reduced renal blood flow, renal failure, and urinary tract obstruction. Patients having kidney dysfunction should periodically check their Cn level [3,4]. In this regard, to develop chemical and biological sensors, efforts have to be made to improve the performance of sensors at the level of both sensing and quantifying trace amounts of Cn. Despite the importance of an accurate quantification of Cn levels, the current methods used for their determination in laboratories and hospitals are complicated and limited, and the interference from other metabolites can lead to an error in the measurement. The clinical laboratory method based on the Jaffe reaction is subject to many interferences and lacks specificity. The Jaffe reaction or the enzymatic colorimetric method is the most common method used for the analysis of Cn, which is affected by numerous metabolites and drugs found in biological samples [5]. To surmount these limitations, the electrochemical technique can be used for Cn determination. It is an attractive method because of its low cost, easy operation, fast response, and a high sensitivity, feasibility, and suitability for real-time detection. Molecularly imprinted polymer (MIP) technique is one of the most convenient methods for the detection of Cn [6]. Cn sensors in which molecular imprinted polymers coupled with electrochemical methods were reported in various works like gold substrates - LAAS-RTB platform, CNRS Toulouse standard silicon technology sensors based on MIP for Cn detection [7]; quantification of Cn in human urine by MIP by Cn binding, a carboxylic polyvinyl chloride (PVC-COOH) layer was functionalized onto screen-printed gold electrodes (Au SPEs) [8]; synthetic receptors for Cn implemented in potentiometric sensors based on the epoxy-graphite matrix as a conductive solid contact [9]; carbon paste electrode modified molecularly imprinted polymer as a sensor for Cn analysis by stripping voltammetry [10] recommends efficiency of MIP-based electrochemical

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sensors Other reported advantages of MIPs are their stability against a wide range of environments and binding affinity, which is comparable to that of biological recognition elements [11–13]. MIPs coupled with electrochemical studies widely accepted mainly because of their features such as easiness of use, and the low cost of realization highlighted their application in the field of electrochemical sensors [14–17]. Graphene oxide (GO) has a large surface area containing a number of oxygen functionalities, including epoxide, hydroxyl, and carboxylic groups. These surface functional groups can provide plenty of reaction sites for the binding molecule of external species. Remarkable electronic transport properties of the sensing material can increase device characteristics such as response, response time, and recovery time. The recent discovery of graphene has led to the revelation of promising materials and sensing qualities [18]. On the basis of the reports so far, graphene is quite a suitable material for all types of chemical sensor applications because it has excellent structural, electrical, and chemical properties. The two-dimensional (2D) nature of graphene increases its suitability for the miniaturization of thin film devices to develop efficient portable sensors with fast response characteristics. The successful combination of GO and an MIP gives a superior route in the application of MIPs in biomolecule sensing [19]. The immobilization of vinyl group on the surface of GO will increase the selectivity [20], and the large surface area and high van der-Waals force makes GO to undergo self-aggregation [21]. GO is attractive in electrochemical sensing because of its large specific surface area (2630 $m^2 g^{-1}$), thus providing the largest sensing area per unit volume, high electron mobility at room temperature, and high sensitivity [22–24]. Owing to the nature of the conductivity of the sensing matrix (either n-type or p-type), the current increases because of the availability of sufficient charged species in GO. Hence, the sensing can be controlled by the cumulative contributions from both MIP and GO, which may increase the device response in many ways. Electrochemical sensing techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are excellent options to combine with MIPs.

In the present work, a Cn sensor, namely silylated GO copolymerized with methyl methacrylate/2-hydroxymethacrylate [TMSPMA-GO-co-HEMA/MMA], was synthesized. Silyl-functionalized GO was prepared by using 3-(tri methoxy silyl propyl) methacrylate (TMSPMA), above which Cn-imprinted polymer was synthesized by polymerization. The pre-polymerization mixture was prepared by using 2-hydroxyethyl methacrylate (HEMA), Cn (template), and methyl methacrylate (MMA). The silylated GO was polymerized with the pre-polymerization mixture, and the resulted polymer chains were grafted onto the surface of the silylated GO to form a strong covalent bonding between the silylated GO and the polymer matrix. Two monomers can effectively interact with two functional moieties in Cn, which could considerably enhance the interaction strength between Cn and monomers. In the first part of grafting, the silyl-functionalized GO reacted with the pre-polymerization mixture in the presence of ethylene glycol dimethacrylate (EGDMA) as crosslinker and 2,2′-Azobis(2-methylpropionitrile)(α,α′-Azo iso butyronitrile, AIBN) as free radical initiator. The physical and chemical properties of the sensor were analyzed by Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy, XRD, scanning electron microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDS) and atomic force microscopy (AFM). Electrochemical studies were conducted by CV and DPV.

2. Experimental

2.1. Reagents

Analytical grade chemicals were used for the investigation. Cn and TMSPMA were purchased from TCI Tokyo Chemical Industry Co. Ltd. Graphite and HEMA were from Alfa Aesar, and MMA was obtained from Central Drug House (p) Ltd. AIBN and EGDMA were purchased from Sigma Aldrich. Potassium permanganate (KM_nO_4) and sodium nitrate ($NaNO_3$) were purchased from E. Merck India Ltd. Conductivity water with specific conductivity less than 1 μ moh/cm was used throughout the experiments in the supporting electrolyte and template solution in the conductivity cell. All aqueous solutions were prepared in duplicate. For analytical applications, the human blood serum and urine samples were collected from a clinical laboratory located at Pothencode near Thiruvananthapuram. The samples were refrigerated at −4 °C before use.

2.2. Instruments for characterization

The concentration of Cn was determined spectrophotometrically using a JASCO UV–visible (model V-530, Japan) spectrophotometer at λ_{max} = 235 nm. The FTIR spectra of the sensor were taken with Perkin Elmer 1800 model IR operating in the 400–4000 cm^{-1} frequency range in transmission mode. Raman spectra were recorded using a micro-Raman spectrometer, Lab Ram UV HR, Jobin-Yvon. XRD patterns of the material were examined using a Siemens D5005 X-Ray unit. Cu K_∞ (λ = 1.54064 Å) radiation generating a voltage of 40 kV and current of 40 mA was used as the X-ray source. SEM analyses were done using an FEI model Nova Nano SEM 450 (USA), provided with a high-resolution FE-scanning electron microscope. Elemental analysis was done using an EDS detector, Bruker model X flash 6/10 (Germany). AFM images were recorded on Bruker DIMENSION Edge with SCAN ASYST instrument in taping mode. All pH measurements were carried out on a Systronics pH meter (μ pH system 362, Systronics India Ltd). CV and DPV analyses were carried out using SP-200 (SN 0437), EC-Lab for windows v10.40 (software).

2.3. Synthesis of Cn-imprinted and nonimprinted polymer materials

A detailed procedure for the synthesis of the sensor: The copolymerization of silylated GO with MMA and HEMA [MTMS-GO-co-HEMA/MMA] is shown in Fig. 1. GO was synthesized from graphite according to the modified Hummers method [25]. For the synthesis of silylated GO, 1.0 g of the prepared GO suspension was added into a four-necked flask filled with deionized water. The mixture was dispersed by ultra-sonication for 2 h and 8.5 mL 10% TMSPMA solution in N,N dimethyl formamide (DMF) was added drop by drop for 1 h, and then, the mixture was stirred for 12 h at 65 °C. Two hundred milliliters of ethanol was then added to remove the residual TMSPMA. The resulting silylated GO was filtered and washed [26]. Cn, MMA, and HEMA were stirred for 1 h in a round bottom flask at room temperature in 35 mL of DMF for the synthesis of the pre-polymerization mixture of template molecule and functional monomers. Approximately 0.6 g of GO-TMSPMA, crosslinker EGDMA, and initiator AIBN (0.92 g) were added to the above mixture; thereafter, N_2 gas was purged under constant magnetic stirring for 12 h at 70 °C. The resultant product was filtered and washed with ethanol to remove unreacted reagents. Warm water was used to remove the template molecule using the Soxhlet extractor apparatus, until the solvent does not show the peak of Cn at 235 nm; the resultant polymer was dried to obtain MIP (TMSPMA-GO-co-HEMA/MMA). The same procedure was used for the synthesis of non-imprinted polymer (TMSPMA-GO-co-HEMA/MMA, NIP). The only difference in the preparation of MIP was the absence of synthesis of the pre-polymerization mixture with template molecule.

2.4. Electrochemical studies

Experimental arrangement for the electrochemical measurements of CV and DPV was done using the electrochemical workstation [SP-200 (SN 0437)]. The sensor capability was checked at 30 ± 7 °C to maintain the temperature. The voltage window was kept between −0.5 and +0.5 to better asses the performance of the sensor, and throughout the experimental work, the voltage was maintained to be less than 20 mV/s, using 50 mM $NaClO_4$ and 5 mM $K_4[Fe(CN)_6]$ as the supporting