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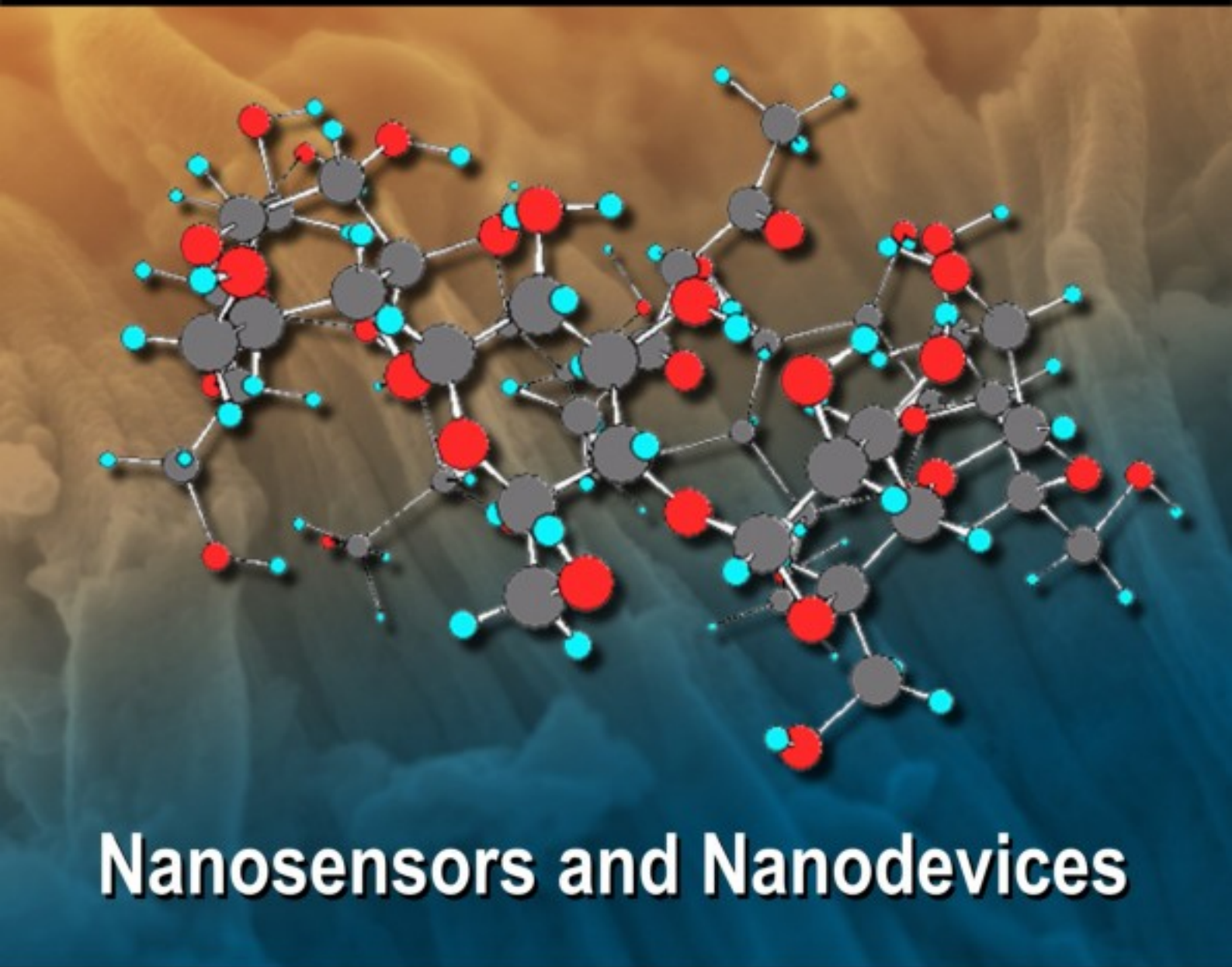

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Electrostatically Functionalized Multi-Walled Carbon Nanotubes Based Flexible and Non-Enzymatic Biosensor for Glucose Detection

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Abstract: We have developed a highly sensitive and selective amperometric biosensor for non–enzymatic glucose detection using electrostatically functionalized multiwalled carbon nanotubes (f-MWCNTs) by thermal embedding technique onto flexible substrates. MWCNTs were functionalized (f-MWCNTs) by electrostatic technique to yield hydroxyl groups on their surfaces. The electrocatalytic activity of the modified electrodes towards glucose oxidation was investigated by cyclic voltammetry and amperometric technique. The f-MWCNTs electrodes showed good electrocatalytic activity towards the oxidation of glucose in 0.1M NaOH solution at an applied potential of 0.4 V. A linear dependence (R= 0.9998) with the glucose concentration up to 11 mM and an extraordinary high sensitivity of 5.63 $\mu AmM^{-1}cm^{-2}$ with a detection limit of 5 µM were observed. The electrocatalytic activity showed a very fast response in less than 2 seconds with a high selectivity, reproducibility over 6 months and negligible response to interfering species such as chloride ions, lactose, ascorbic acid, fructose and uric acid. The fabrication of the flexible f-MWCNTs electrodes requires low cost, provides long term stability and re-usability and can be easily developed for enzyme-free glucose sensors. *Copyright © 2012 IFSA.*

Keywords: Carbon nanotubes, Biosensor, Electrostatically, Corona discharge technique.

1. Introduction

Biosensors are important and essential devices in the present day-to-day clinical applications and environmental monitoring of pathogens and toxins [1-3]. Therefore, the development of highly

sensitive, low-cost, reliable glucose sensors with an excellent selectivity have been the subject of research for decades, not only in medical science but also in the food industries [4, 5]. Numerous glucose biosensors have been developed for glucose level monitoring since the first glucose biosensor was developed by Clark and Lyons in 1962 [6]. Turbidity sensors [7], viscometric affinity sensors [8], fluorescence based sensors [9], and electrochemical biosensors have been extensively studied [10, 11]. Among these, the electrochemical biosensor can be a good choice because of its simplicity, rapid response, high sensitivity, and low cost. However, most of the methods mentioned above for the detection of glucose are enzyme based. The first amperometric enzyme glucose sensor was developed in 1973 [12], in which the anodic production of hydrogen peroxide was analyzed instead of the highly variable oxygen reduction current. There are some disadvantages of the enzyme- modified electrodes, such as instability, high cost of enzymes, complicated immobilization procedure, critical operating situation, etc.

The key aspect of an electrochemical biosensor is the generation or modulation of electrical current in an electronic circuit between the bio-reaction or bio-recognition systems and the electronic elements. The high demand for selection and selectivity requires not only the appropriate biological macromolecules with high active, but also the suitable substrates with biocompatible surroundings and efficient transport of electrons. Keeping this in view, the recent developments in the nanotechnology have paved the way for a large number of new materials and devices of desirable properties which have useful functions in numerous electrochemical sensor and biosensor applications [13-17]. Since the discovery of carbon nanotubes (CNTs) by Ijima in 1991 [18], multiwall carbon nanotubes (MWCNTs) have been utilized for fabrication of electrochemical biosensor [19–22]. CNTs, the graphite allotropes of carbon, are by far the most widely used nanomaterials for the fabrication of electrodes due to their semi- conductive behavior, unique optical, electronic, mechanical, and chemical properties, high porosity, elevated electrochemical activity and eminent electron communication features.

Usually, nanotubes and nanowires are incorporated into the functional systems by a variety of methods, such as solution evaporation, sol-gel encapsulation, and polymer assisted dispersion. These methods generally results in disarrayed and layered films with low amperometric response upon bio-electrocatalysed oxidation or reduction of the analyte. To overcome this problem, perpendicularly aligned nanotubes or nanowires arrays can be formed as sensing devices [23, 24], with an increment of enzyme content associated with the electrode surface, an improvement of electrical communication and thus an enhancement of the transducer amperometric signal. ITO is currently the most popular material used for the electrode fabrication of these sensing devices but it has a number of deficiency. It is not very compatible with biological materials, unfavorable mechanical properties such as relatively fragile and is relatively very costly. Therefore, developing and commercializing a replacement for ITO is a major focus of our research. Devices based on plastic substrates represent the next technological challenges to reduce the weight and cost and keeping this view in mind we have fabricated our film on a flexible, low cost, non conducting parafilm. The construction and performance of a novel colloidal gold-CNT composite electrode using Teflon as the non-conducting binding material was reported by Manso et al.2007 [25].

The major problem on the promising applications of CNTs in electrochemical sensors is the immobilization of activated CNTs on the electrode surface because CNTs generally exist as highly tangled ropes and are insoluble in almost all solvents, which greatly hinder their capacity of forming uniform and stable films. To overcome this deficiency, CNTs are firstly dispersed or dissolved in various solutions or suspensions and immobilized on the surfaces of various substrates by physical or chemical methods. This section focuses on the introduction of some typical immobilization methods of CNTs on electrode surfaces that are widely used in constructing CNT-based electrochemical sensors.

Herein, we report a study on nonenzymatic detection of glucose with electrostatically functionalized MWCNTs with oxygenated functional groups and transferred onto flexible polyfilms by thermal embedding technique. We fabricated highly sensitive, selective, stable and fast amperometric glucose sensors operating at 0.1 M NaOH solution. The electrostatic functioanlization was developed by Bhatia et al. [26] and provided a uniform functionalized surface of MWCNTs with oxygenated functional groups surface to promote the faster electron transfer for the glucose oxidation reaction via the MWCNTs. We also demonstrated that the electrostatically functionalized MWCNTs electrodes were insensitive to interfering agents such as chloride ions, lactose, ascorbic acid, fructose and uric acid. The working electrode developed when tested as a non enzymatic glucose sensor shows high sensitivity, selectivity, reusability and stability.

2. Experimental Section

2.1. Reagents and Materials

MWCNTs were purchased from sigma and were used as received. d-(+)-Glucose, dopamine (DA), l-ascorbic acid (l-AA), uric acid (UA), d-fructose, lactose and sucrose were purchased from Qualigens and were used as-received. Deionized water (>18.4M_cm−1) was used for all solutions' preparation. All other reagents were of analytical grade and used without further purification. The electrochemical measurements were performed in 0.10 M NaOH solution with three electrode system including f-MWCNTs as a working electrode and an Ag/AgCl (3MKCl) electrode and a platinum wire as reference electrode and counter electrode, respectively. The glucose stock solution was allowed to mutarotate at least 24 hrs before use.

2.2. Functionalization of MWCNTs

A new technique to functionalize the thin films of carbon nanotubes with oxygenated functional groups with electrostatic charging method, developed by Bhatia et al. [26], was implemented to functionalize the MWCNTs in this study. During the electrostatic functionalization of MWCNTs, due to generation of high intensity electric fields, atmospheric oxygen was ionized to highly reactive native oxygen which was adsorbed on the surface of MWCNTs and generated oxygenated functional bonds on the surface. The functionalization of the films, f-MWCNTs was confirmed with Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy [26].

2.3. Fabrication of the Electrode for Electrochemical Studies

The electrodes of f-MWCNTs were fabricated by self-assembly of f-MWCNTs by thermal embedding onto parafilm. 5 mg of f-MWCNTs were dispersed in 10 ml of ethanol and sonicated for several hours to provide a uniform suspension. The dispersed solution of f-MWCNTs was cast on cleaned glass surface and allowed to dry under ambient conditions for several hours. The cast f-MWCNTs were then self-assembled on to the surface of non-conducting parafilm by thermal embedding technique by heating at oven temperatures for a few minutes to achieve a desired thickness of about 100 μm of the film. After self-assembly f-MWCNTs on to the parafilms, thin strips of 1mm×1mm were cut from the parafilm for using them as electrodes. Electronic grade silver paste was used for wire bonding with the strips. Fig. 1 provides the photographic illustration of the fabrication steps involved in fabricating f-MWCNT electrodes onto parafilm. The morphology of the f-MWCNTs was investigated by field emission scanning electron microscopy (FESEM) Zeiss EV040 as shown in Fig. 2 (a) and transmission electron microscopy (TEM) JEOL 2100F as shown in Fig. 2 (b).

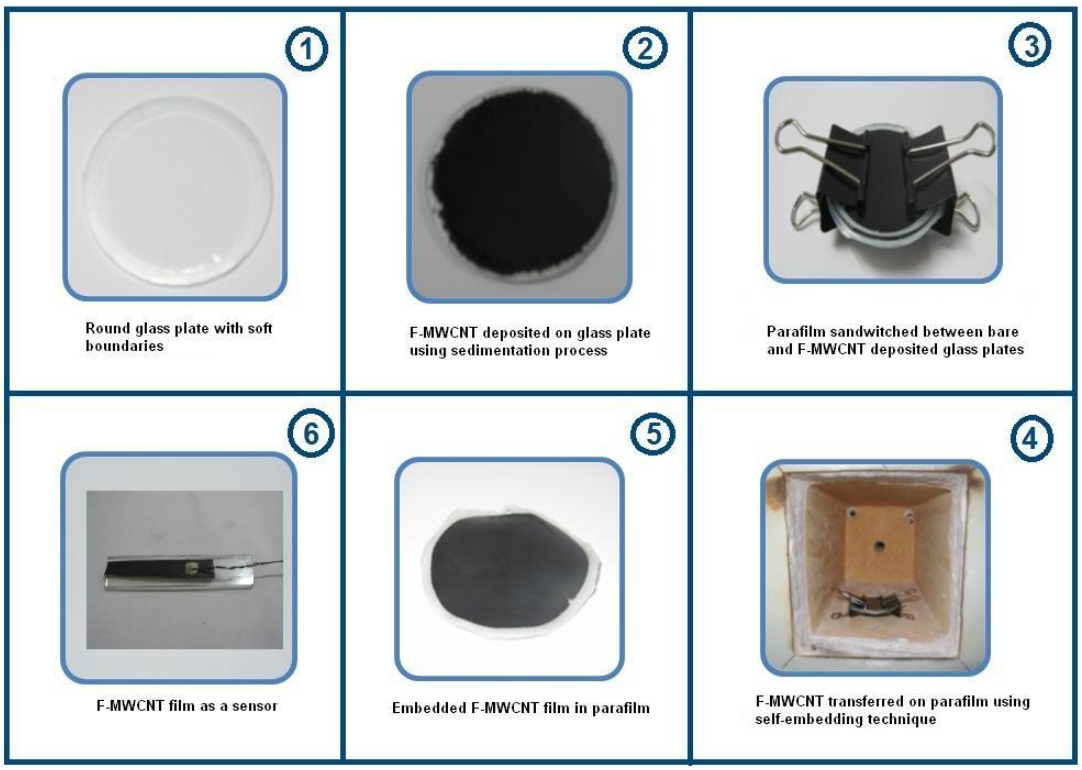
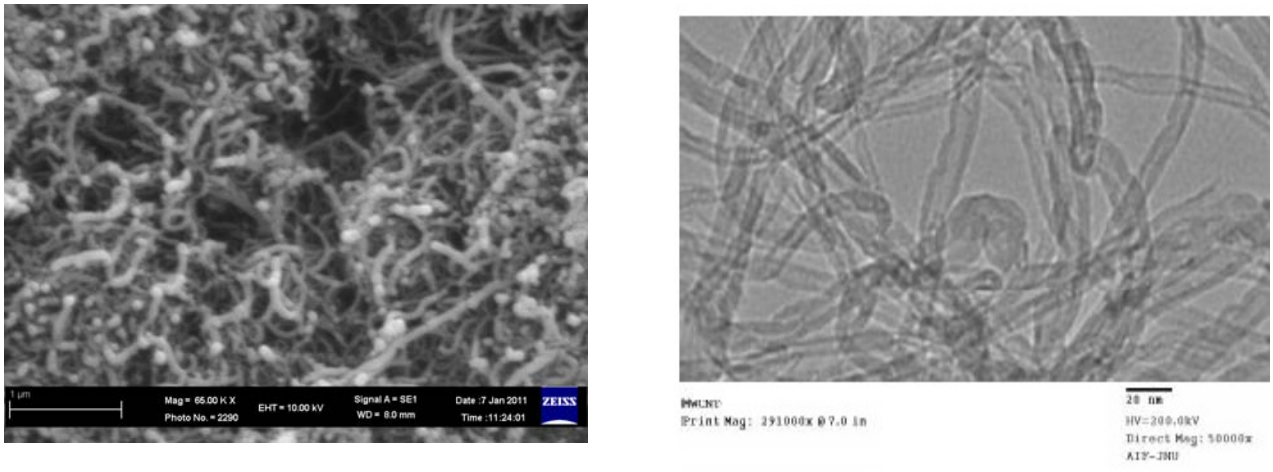


Fig. 1. 1-6 illustrates consecutive images of steps involved in fabricating strips of self- assembled f-MWCNTs onto parafilm by thermal embedding technique.



(a) (b)

Fig. 2. Field emission scanning electron microscopy (FSEM) image of electrostatically f- MWCNTs (a); Transmission electron microscopy (TEM) images of f-MWCNTs (b).

2.4. Experimental Measurement Procedure

Electrochemical experiments were performed using Gamry electrochemical workstation (Gamry Instruments, USA) with a conventional three-electrode setup, including f-MWCNTs as a working electrode and an Ag/AgCl (3MKCl) electrode and a platinum wire as reference electrode and counter electrode respectively. The electrolyte solutions were purged with high purity nitrogen for at least 30 minutes prior to each electrochemical measurement and the nitrogen environment was maintained

over the electrolyte to protect the solution from oxygen. All the measurements were performed under a continuous magnetic stirring at 250 rpm of 5 ml electrolytic solution to provide convective mass transport at the electrodes. It was observed that higher speeds of stirring led to an increase in noise while a lower speed resulted in a longer response time In order to improve the electrocatalytic performance of the working electrode, various influencing factors including the applied potential and the concentration of NaOH were investigated. The maximum response of current and a good signal/noise ratio were observed at 0.4 V. Amperometric detection was performed under an applied potential of 0.4 V. The response was taken as the change between the steady state and background currents. All electrochemical experiments were performed at room temperature (23 ± 2 ºC).

3. Results and Discussion

3.1. Electrocatalysis of Glucose at the Electrostatically f-MWCNTs Electrode

The electrocatalytic activity of the electrostatically f-MWCNTs composite towards the oxidation of glucose in an alkaline solution was demonstrated. Fig. 3 shows the cyclic voltammograms (CV) of the f-MWCNTs electrode in a 0.1 M NaOH solution with glucose in the range of 2, 4 and 6 mM glucose at a scan rate of 50 mVs^{-1}. In the alkaline solution, the CV showed the oxidation process starts at ~ +0.10 V with a shoulder peak at ~ +0.42 V indicating a strong catalytic function of the f-MWCNTs towards the direct oxidation of glucose as well as an improved reversibility of electron transfer processes at f-MWCNTs electrode.

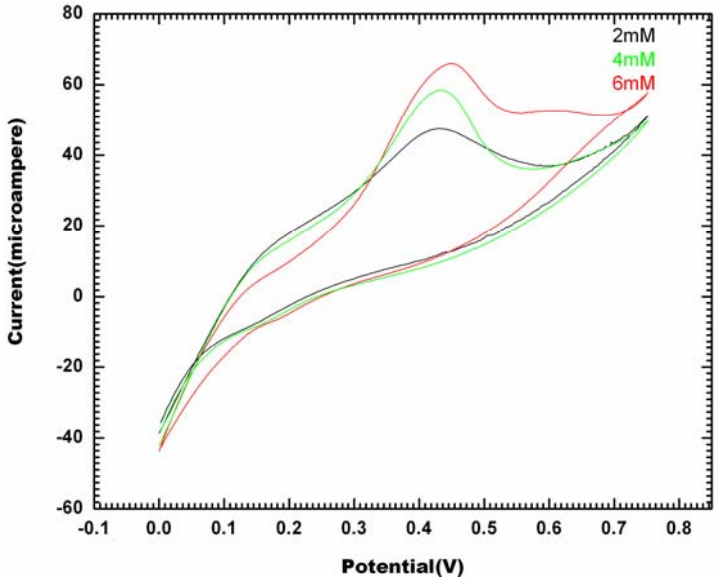


Fig. 3. Cyclic voltammetric response of the f-MWCNTs electrode in 0.1 M NaOH solution in the presence of 2 mM, 4 mM and 6 mM glucose at a scan rate of 50 mV/s.

Fig. 4 illustrates the range of concentration of glucose tested with f-MWCNTs at 0.4 V. The limit of detection was estimated to be 0.5 µM at a signal to noise ratio of 3. The electrode displayed a linear range of 0.5 µM to 11 mM glucose with a correlation coefficient of 0.99986. The slope of the calibration curve provided the glucose sensitivity to be of 5.63 μAmM^{-1} cm^{-2}. The results indicated that the electrochemical kinetics is controlled by the adsorption of glucose [27]. The upper- limit of the linear range is far above the physiological level (3-8 mM) which suggested that the electrode developed here would be useful in the determination of the glucose concentration in physiological levels.

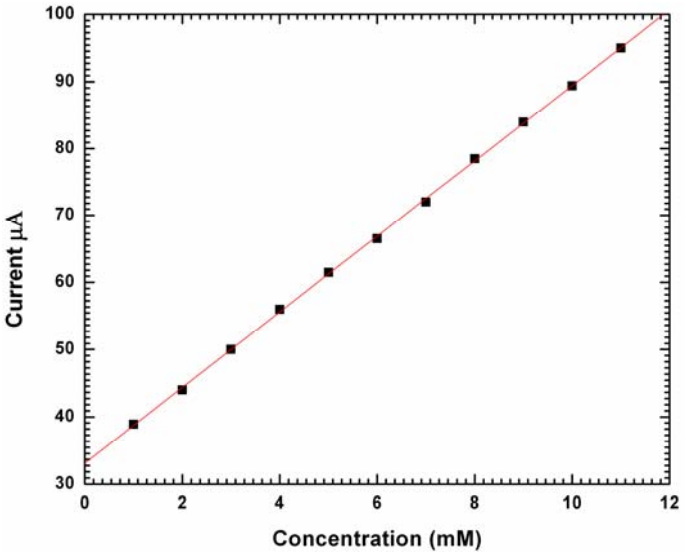


Fig. 4. The calibration curve of current vs. concentration of glucose at the f-MWCNT's electrode.

3.2. Amperometric Response of Interfering Species towards f-MWCNTs Electrode

The normal physiological level of glucose is 3-8 mM, which is much higher than those of interfering species of AA, AP, UA and other carbohydrates which normally co-exist with glucose in real samples. Since the interfering species have higher electron transfer rates than glucose, their oxidation currents are comparable to that of high concentrated glucose. The amperometric response of f-MWCNTs electrode towards the addition of 4.0 mM glucose and 0.05 mM AA, UA, DA and other carbohydrates in 0.1 M NaOH as shown in Fig 5. The result indicated that the current response of the interference was only 0.03-0.22 % of that of the glucose at the MWCNT's electrode. Therefore the selectivity of the electrode for the glucose detection was satisfied at the presence of possible interfering reagents.

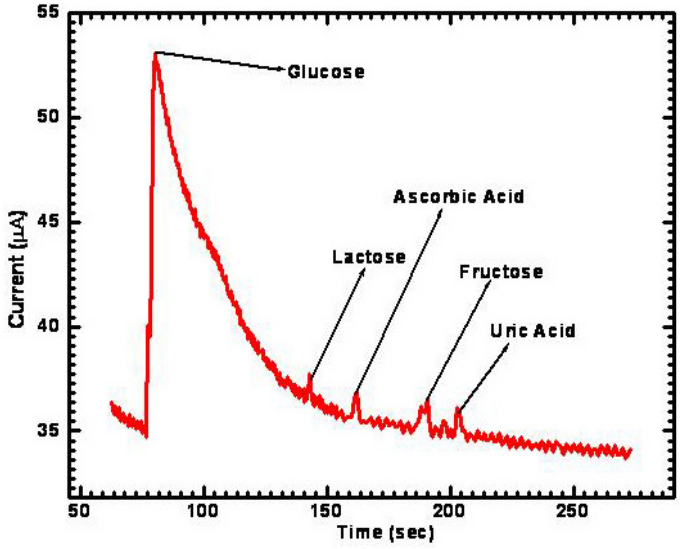


Fig. 5. Flow injection amperometric response of f-MWCNTs electrode to injection of 4mM glucose and 0.05 mM other interfering species.

3.3. Stability Response of f-MWCNTS Electrode

The response of electrode towards 7 mM glucose was recorded at an interval of 1 month and is represented in Fig. 6. The response revealed that the electrode maintained nearly the same initial current response as when fabricated towards the glucose during the duration of 6 months. This indicated that electrode can be used as a stable electrode for glucose detection over duration of 6 months.

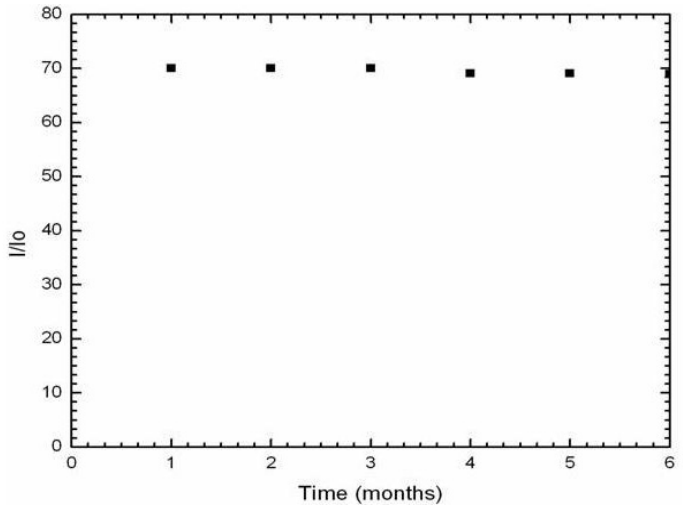


Fig. 6. Performance of the f-MWCNT electrode towards 7 mM glucose over an interval of 6 months.

3.4. Fouling by Chloride Ions

Most of the electrochemical glucose sensors based on metal [28] or alloys [29] can easily lose their activity due to the poisoning of chloride ions. In order to understand poisoning by chloride ions, the current response of f-MWNTs electrode was examined by adding 0.15 M NaCl in the 0.1 M NaOH electrolyte. The experimental results, as shown in Fig. 7, indicated that there was no current response of the electrode in presence of Cl^- ions, indicating that the electrode was not poisoned by Cl^- ions.

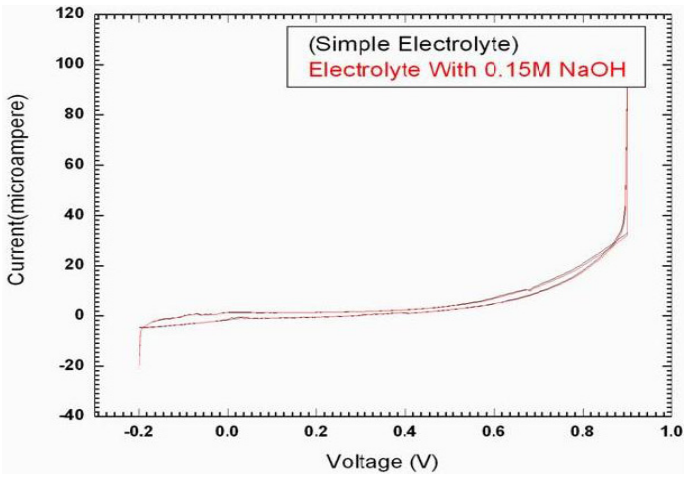


Fig. 7. CV of f-MWCNTs electrode in the presence and absence of NaCl.

4. Conclusions

Electrostatically functionalized MWCNT were fabricated by a facile and effective method on a flexible substrate by self embedding techniques. The electrochemical studies revealed that the electrodes exhibit good electrocatalytic responses to glucose in alkaline media 0.1 M NaOH with a number of attractive features such as high sensitivity, selectivity, stability, good reproducibility and fast response. The electrode provided a fast response time (<2 s) and a linear dependence (R=0.9998) in the glucose concentration range of 0.5 μM to 11 mM with a very high sensitivity of 5.63 $\mu AmM^{-1}cm^{-2}$ at an applied potential of +0.4 V. Furthermore, the sensor can effectively resist the effect of interferences. The electrode fabricated by such a convenient and effective process may be used as non-enzymatic glucose sensor materials for routine analysis of glucose in real blood serum samples or in fermentation industry.

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Sensors & Transducers Journal

Guide for Contributors

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Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because of it is a peer reviewed international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per year by International Frequency Sensor Association (IFSA). In additional, some special sponsored and conference issues published annually. *Sensors & Transducers Journal* is indexed and abstracted very quickly by Chemical Abstracts, IndexCopernicus Journals Master List, Open J-Gate, Google Scholar, etc. Since 2011 the journal is covered and indexed (including a Scopus, Embase, Engineering Village and Reaxys) in Elsevier products.

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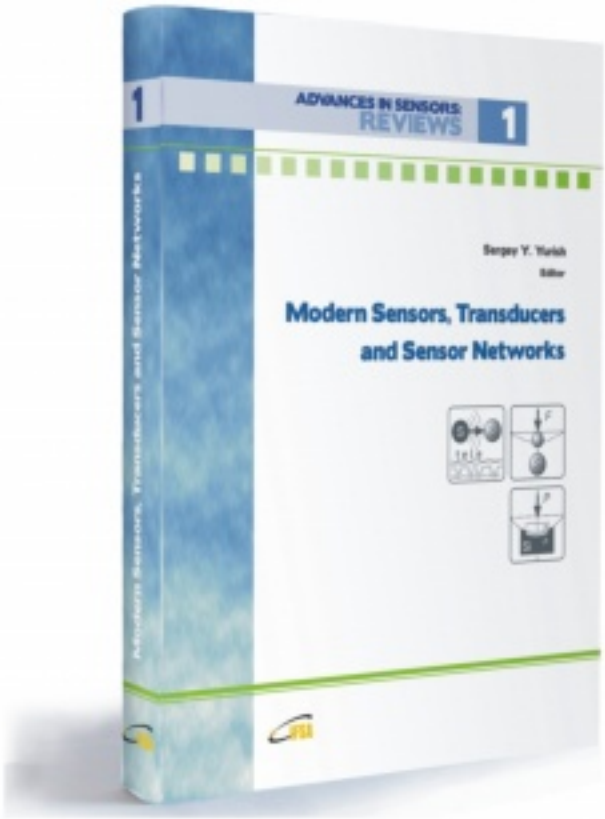

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