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Phenylboronic acid functionalized reduced graphene oxide based fluorescence nano sensor for glucose sensing

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ABSTRACT

Reduced graphene has emerged as promising tools for detection based application of biomolecules as it has high surface area with strong fluorescence quenching property. We have used the concept of fluorescent quenching property of reduced graphene oxide to the fluorescent probes which are close vicinity of its surface. In present work, we have synthesized fluorescent based nano-sensor consist of phenylboronic acid functionalized reduced graphene oxide (rGO–PBA) and di-ol modified fluorescent probe for detection of biologically important glucose molecules. This fluorescent graphene based nano-probe has been characterized by high resolution transmission electron microscope (HRTEM), Atomic force microscope (AFM), UV–visible, Photo-luminescence (PL) and Fourier transformed infrared (FT-IR) spectroscopy. Finally, using this PBA functionalized reduced GO based nano-sensor, we were able to detect glucose molecule in the range of 2 mg/mL to 75 mg/mL in aqueous solution of pH 7.4.

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1. Introduction

Reduced graphene has emerged as promising tools for detection based application of biomolecules. Graphene oxide is a two-dimensional sheet like nanostructured carbon based nanomaterials. It is composed of mainly sp^3 and sp^2-hybridized carbon atoms with various functional groups like –COOH, –OH and epoxide on their surface. Whereas Graphene, a single-atom-thick monolayer containing sp^2 carbon atoms present in conjugated form in a sheet like structure. Graphene has high surface area with high electronic conductivity and strong fluorescence quenching property which is being used for electrical [1] and optical [2] detection of various biomolecules. D-Glucose is an elementary necessity of living creatures and a ubiquitous bio-fuel for many biological practices. Glucose level is maintained by its own biological process. Abnormality of glucose level in blood leads to disease like diabetes. Therefore sensing and determination glucose level in blood has become emerging field for the diagnosis of diabetes. Many methods have been published on sensing of glucose molecules like electrochemical sensing [3–6], Quantum dots immobilized microgel based optical sensor [7], droplet-based microfluidic electrochemical sensor [8], photonic crystal based naked eye glucose sensing [9], liquid crystal based glucose sensing [10], aggregation induced fluorescent enhancement and quenching based sensing [11,12], and many other enzymatic [13] and non-enzymatic based glucose sensing [14–18]. Detection of glucose using phenylboronic acid functionalized various scaffolds has been reported [3,12,19–22]. A few papers have shown fluorescence based glucose sensing using graphene oxide based nano probe [23–25]. Preparation of graphene oxide based enzymatically electrochemical glucose sensor are costly as well as handling of enzyme is not easy. Electrochemical catalytic activity of graphene based for electrochemical sensing of glucose is poor. Therefore for better catalytic activity graphene based materials are made composite with other metals or metal alloys nanoparticles [4,6,15,17]. A few papers have been reported for glucose detection using fluorescent functionalized graphene quantum dots [11,23]. But in this case, further chemical modification of graphene based materials is needed for fluorescent graphene quantum dots preparation. Hao Zhang et al. reported turn on fluorescence based sensor for glucose determination using graphene oxide–DNA scaffold [24]. Therefore development of properly affinity molecules functionalized graphene oxide based non enzymatic and fluorescence based glucose sensor need to be explored for the sake of scientific community especially those who are working in the field of bio medical science. Here, in this paper we have used phenylboronic acid functionalized reduced graphene oxide (rGO–PBA) for fluorescence based detection of glucose. We developed an easy and simple cost-effective synthetic method for preparation of PBA functionalized reduced graphene oxide based nano probe. Finally, using this nano probe glucose has been detected in the range of 2 mg/mL to 75 mg/mL at pH 7.4. It's a fluorescent based enzyme-free detection of glucose in solution.

In this work, we used the concept of fluorescent quenching property of reduced graphene oxide of a fluorescent probes which are close vicinity of its surface. Phenylboronic acid is well known for having preferential affinity to diol containing molecules through cyclic ester bond formation. Therefore we prepared a diol modified fluorescent probe for specific interaction with phenylboronic acid. That's why, we have used phenylboronic acid functionalized reduced

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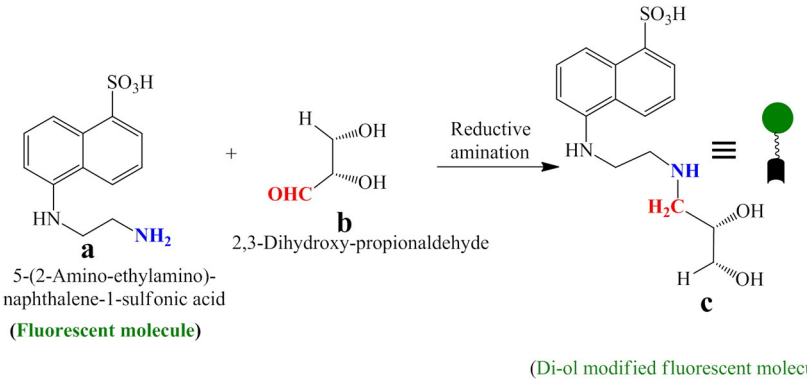


Scheme 1. Synthesis of di-ol modified florescent probe (c) starting from 5-(2-Amino-ethylamino)-naphthalene-1-sulphonuc acid (ANSA) (a) and 2,3-dihydroxy-propionaldehyde or glyceraldehyde (b) by reductive amination method.

graphene oxide (rGO–PBA) and di-ol modified fluorescent probe (Scheme 1-c) for fluorescent based detection of glucose molecule. First, 3-aminophenylboronic acid was conjugated to graphene oxide surface followed by the hydrazine reduction and a fluorescence molecule (5-(2-Amino-ethylamino)-naphthalene-1-sulphonuc acid) was conjugated to the glyceraldehyde by reductive amination method to synthesize di-ol modified fluorescent probe (Scheme 1-c). On addition of diol modified fluorescent probe to the PBA functionalized reduced GO solution; phenylboronic acid on the reduced graphene oxide forms a cyclic boronate ester with fluorescent probe. Therefore, fluorescent probe come close to the reduced GO surface and hence the fluorescence is quenched (Scheme 2). Upon addition of glucose molecule to the quenching state of di-ol modified fluorescent probe in the cyclic ester form with PBA of the rGO–PBA, glucose forms new cyclic boronate ester with rGO–PBA and replace the di-ol modified fluorescent probe from the reduced GO surface. As a result fluorescence property of the di-ol modified fluorescent probe comes back from its quenched state. In this non-enzymatic and cost effective approach, the PBA functionalized reduced graphene oxide (rGO–PBA) fluorescence based sensor has been used to detect glucose molecule in the limit of 2 mg/mL to 75 mg/mL.

2. Experimentals

2.1. Materials

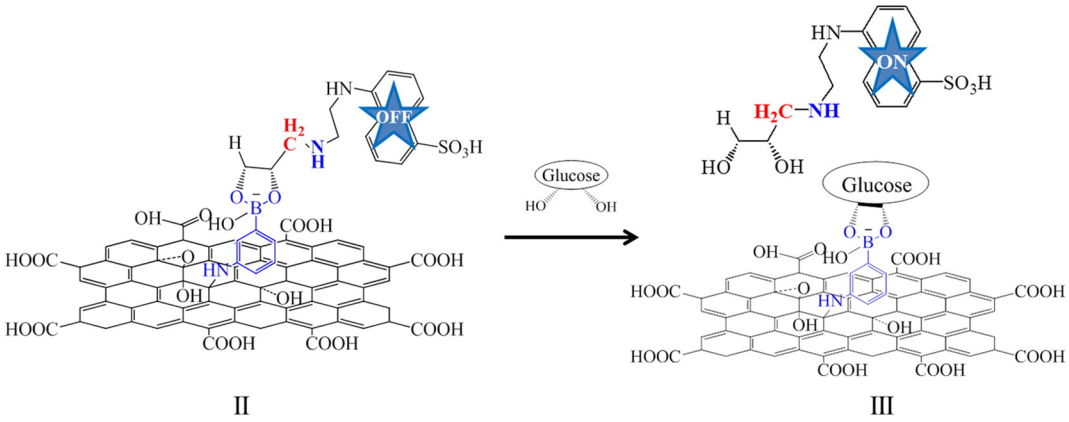
Graphite powder, 3-aminophenylboronic acid (APBA) (MW 136.96), glyceraldehyde, glucose, dextran (MW 6000), 5-(2-Amino-ethylamino)-naphthalene-1-sulphonuc acid (ANSA), hydrazine monohydrate, NH_3 solution, Alizarin red S. (ARS) all were purchased from Sigma-Aldrich and used as received.

2.2. Instruments

The UV–vis absorption spectra were recorded on a Shimadzu UV-2550 UV–vis spectrophotometer, and the fluorescence measurements were performed on a BioTek Synergy Mx microplate reader. High resolution transmission electron microscopy (HRTEM) images were obtained using a JEOL-JEM 2010 electron microscope. Atomic force microscope (AFM) was measured using VEECO DICP II autoprobe (model AP 0100). Fourier transform infrared (FTIR) spectra of KBr powder-pressed pellets were recorded on a Perkin-Elmer Spectrum 100 FTIR spectrometer.

2.3. Synthesis of graphene oxide (GO)

Graphene oxide was synthesized by modified Hammer's method [26]. Typically, 200 mg graphite powder, 100 mg sodium nitrate and 5 mL concentrated H_2SO_4 were mixed together in a 100 mL beaker and cooled to 0 °C. Then the solution was kept under vigorous stirring. Next, 600 mg $KMnO_4$ was added to this solution in stepwise manner so that the temperature was should not raise above 20 °C during these $KMnO_4$ addition steps. After the complete addition of $KMnO_4$ the temperature of the solution was slowly raised to 35 °C and kept in this condition for 30 min. A brownish gray paste was formed. Next, 10 mL water was added to the whole solution and the solution turned brownish yellow. The temperature of the solution was increased to 98 °C during water addition and this temperature was maintained for 15 min. The whole solution was then mixed with 28 mL of warm water followed by addition of 500 mL 3% H_2O_2 that reduces the residual permanganate. The light yellow particles were washed thoroughly with warm water



Scheme 2. Fluorescence based sensing of glucose molecule using PBA functionalized reduced graphene oxide (rGO–PBA). Here OFF stands for no fluorescence signal and ON stands for strong fluorescence signal from the solution in presence UV light.

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