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Detection of Biomolecules in High Ionic Strength physiological Solution Using Electrochemically Reduced Graphene oxide Field-Effect Transistor Immunosensor

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Abstract
Graphene has capability for electrical biosensing due to its two-dimensional nature and high carrier mobility. In electrochemically reduced graphene oxide (ERGO) based field-effect transistor (FET), conductivity has been change to a great extent. Low noise FET biosensors have been fabricate with electrochemically reduced graphene oxide(ERGO) on fluorine doped tin oxide(FTO) glass, directly by electrochemical reduction of GO in solution. It has been observed that the sensor is capable of detecting down to1 fM concentration of Hepatitis-B in serum. Detecting charges and the sensitivity of the devices in high ionic strength solutions suffers from the ionic screening due to mobile ions present in the solution and detection of target molecules is a challenge due to the inherent problem of ionic screening in small Debye length. Here we successfully demonstrate that the fundamental ionic screening effect can be mitigated by using smooth ERGO-FET as a high-frequency biosensor. The charge transfer probability between the biomolecules and the surface even at high ionic strength of the buffer have been raises and these features coupled with high frequency sensing have enabled detection down to 1 fM Hep-B in a high ionic strength buffer (150mM) at 8 MHz.
Keywords: ionic screening; Debye length; high frequency; electrochemically reduced graphene oxide(ERGO); high frequency

Introduction: GRAPHENE is a two-dimensional array of carbon that is covalently connected via sp2 bonds to form a honeycomb sheet [1]. Owing to the presence of abundant functional moieties and high electron mobility, it is considered as an attractive platform for various sensing applications [2], [3]. Graphene exhibits a honeycomb structure. Its numerous interesting properties like high electron mobility, room temperature quantum hall effect, tunable optical properties and high mechanical strength have triggered the various application of graphene in biosensors, nano electronics, batteries, super capacitors, gas sensors, pH sensors and hydrogen storage [4-6]. Also, graphene is chemically stable in aqueous solution owing to its wide potential window. These features make graphene field effect transistors (G-FETs) attractive for use as electrical biosensors. For the fabrication graphene based biosensors different types of graphene reduction and deposition processes have been used on various substrates like, direct electrochemical deposition of GO [7,8], drop- casting of graphene [9,10], drop-casting of GO followed by its electrochemical reduction [11] and also reduction by hydrazine [12], spray-coating [13] and spin-coating [14], have been mostly used. The thickness and uniformity of graphene films are not well controlled by these methods which deteriorate the stability and reliability in FET bio sensing. Compared to all the graphene reduction methods mentioned here, electrochemical reduction has become popular received in terms of FET biosensing applications. The greatest advantages of electrochemical reduction over other methods are mild reaction conditions, control of the reduction process, achieving a complete reduction of graphene [15].
However, there is a significant limitation in electrical bio sensing, that is, Debye screening. In aqueous solution, the sensor and target surface are electrically shielded by an electrical double layer with a particular characteristic length is called the Debye screening length. Debye screening is particularly significant in G-FETs, where no insulator exists and zero distance can be achieved between the graphene layers and the target molecules.
However detecting charges in high ionic strength solution is a challenge due to the inherent problem of ionic screening in small Debye length. Even electrophoretic mobility of ions reduces as ionic strength is increased, thereby lowering the capture of target molecules [16]. To overcome this effect, high frequency signal has been applied in single walled carbon nanotube structures which have enabled detection of biotin in presence of 100mM buffer solution [17]. But the limit of detection achieved has not been satisfactory. Furthermore, another concept has been introduced for enzymatic assay using G-FETs. Although the electrical detection of enzyme products using pH sensor and other devices has been reported, [18-21]. For the electrical bio sensing only enzymatic products have been requires near the sensor surface. Therefore, the target was encapsulated in a femto liter micro droplet to accumulate its reaction products around a G-FET and this concept also used in optical single molecule detection [22-

25] and overcome the limitation of Debye screening by developing a bio sensing platform consisting of a G-FET and a microfluidic structure. However, the limit of detection is not state-of-the art for clinical diagnostics.

Fragmentation and size reduction of the bioreceptor (antibody) molecules have been effectively implemented to overcome the screening effects associated with the sensing of biomolecules under high ionic strength conditions by permitting the bio recognition events to occur in closer proximity to the nanowire surface, falling within the charge sensitive Debye screening length [26]. But the overall fragmentation pattern observed for an antibody is a complex result of structural and solvent conditions and some bonds are prone to cleavage due to the presence of specific side chain, leading to a poor detection limit.

To reduce the limit of detection in high ionic strength buffer, we have reported high frequency sensing using smooth graphene FET immunosensor. Graphene structure is expected to enhance the sensitivity owing to certain reasons. Firstly, the heterogeneous charge transfer gets facilitated and the electrode potential reaches equilibrium faster for a given electrochemical system [27]. Secondly, the size dependent effective energy gap of graphene structure increases the transconductance of the device [28]. Thirdly, due to the absence of edges, every section of the device contributes to the conductance of the fabricated smooth graphene layers structure and hence all the antigen molecules that get captured at any location have an impact on the change in conductance [29]. Also there is a probable localization effect of the charge modulation after antigen capture in the graphene structure, which can result in an enhanced change of the overall carrier transport [30].

In this paper, we have superimposed a high frequency ac signal in the range of 10 KHz to 15MHz across the gate and source terminal in an ERGO-FET structure for detection of Hepatitis-B in femtomolar concentration.

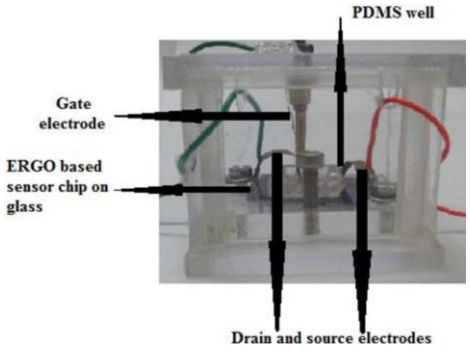
2. Method and Material:

2.1 Fabrication of electrochemically reduced graphene oxide (ERGO): Modified Hummer's method has been used for synthesized GO from fine graphite powder and the ERGO film have been electrodeposited on FTO coated glass substrate through three electrode system. Here the GO has been electrochemically reduced on FTO coated glass substrates by chrono-amperometric technique at room temperature (25◦C). In this fabrication process, the specially treated GO solution has been transferred into a three electrode cell where FTO coated glass substrate (1 cm × 1 cm), a platinum wire and Ag/AgCl (3 M) have been used as working, counter and reference electrodes respectively. The deposited black ERGO films on FTO coated glass substrates have been rinsed thoroughly with deionized water (DI) for several times and dried in vacuum oven for two hours at 60◦C.

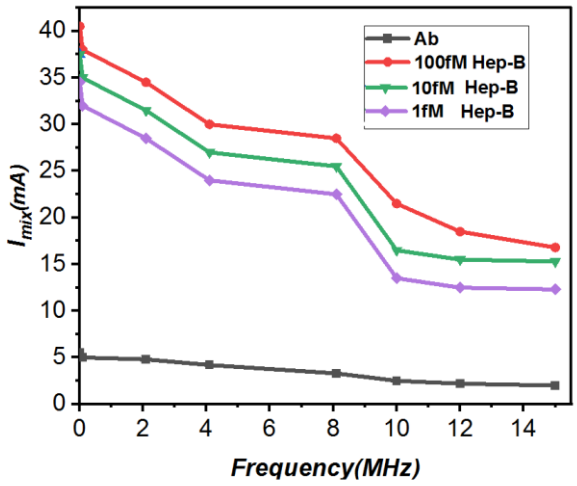
2.2 Functionalization of ERGO-FET — Metal electrodes have been fabricated on ERGO-FTO substrate by screen printing of high temperature silver paste, followed by annealing at 720◦C. The width and length of the electrodes are 100 μm each. For immobilization of anti-Hepatitis-B monoclonal antibody, (procured from Sigma Aldrich, St. Louis, MO, USA) the ERGO coated sensor has been treated with standard N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)-N-hydroxysuccinimide (NHS) chemistry discussed in details in [31]. This process activates the negatively charged carboxylic groups of the RGO and improves the covalent binding of the antibodies. Different concentrations of Hep-B solution in the range of 1fM to 10pM have been prepared by serial dilutions with 150 mM phosphate buffer saline (PBS, pH 7.4). This is a standard technique for preparing low concentration solution [32]. To enclose the active region of the sensor, a well of approximately 2 mm by 2 mm dimension has been cut using a diamond scriber in a polydimethylsiloxane (PDMS) sheet of 2.5 mm thickness. Then the well has been placed on the substrate by observing under an optical microscope (Leica DM 2500). The width of the PDMS sheet is around 4 mm on all the sides. The PDMS sheets made from SYLGARD 184A and SYLGARD 184 B (Product code: 761036 and 761028 respectively) has been procured from Sigma-Aldrich. For proper sealing of the PDMS with substrate, oxygen plasma treatment has been done. During testing process, a PDMS cover has been placed on top of the well after filling it with electrolyte, to reduce any possible fluctuations in the drain current due to evaporation.

2.3 FET measurement — Sensor chip has been interfaced with the PC through a sensor holder set up. In the setup, the bond pads (fabricated on the substrate) of the sensors have been placed below the probes of the sensor holder.

The probes of the sensor holder which are in contact with the drain and source electrodes are spring loaded and an adjustment screw has been provided to align them with the bond pads. The gate electrode has been inserted through a small hole in the setup and soldered to a metal connector. The picture of the setup is shown in Fig. 1. An AC voltage has been fed between the source-gate terminals with the frequency range of 10 KHz to 15 MHz have been applied in drain terminals. Drain-source current measurements have been performed using a Keithley 6487 at room temperature. Hepatitis-B has been selected as the antigen and its different concentrations have been prepared by serial dilution using 150mM buffer. In pH 7.2, Hepatitis-B is negetively charged [33] and has been dispersed onto the sensor platform. After 10 minutes, the surface has been thoroughly washed to remove any non-specifically bound antigen and the current readings have been recorded again in the buffer for measurement of sensitivity.

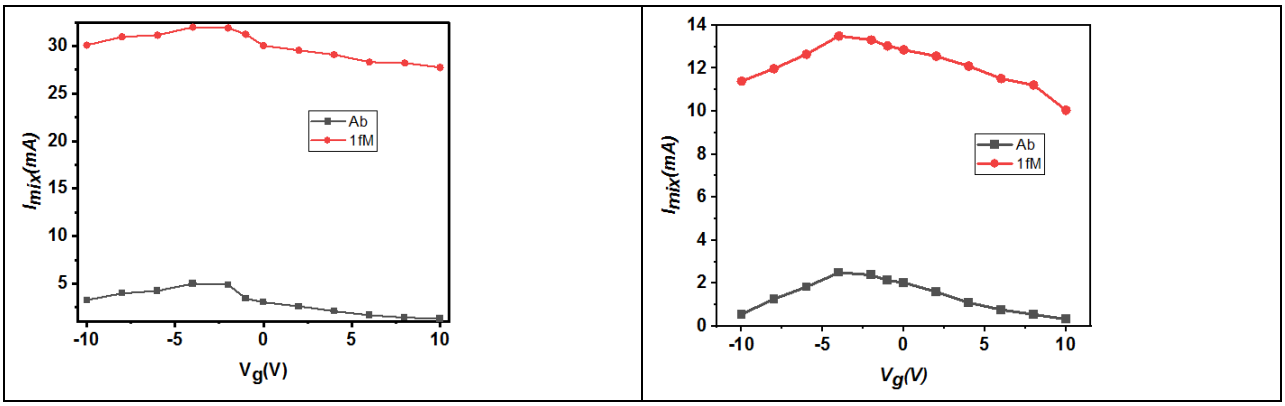


3. Results and Discussions: I_{mix} has been measured both after antibody immobilization and Hep-B capture, as shown in Fig.2. It has been observed that after antibody attachment I_{mix} decreases with frequency in 150mM buffer but decreasing rate is not rapid in nature. In case of Hep-B attachment I_{mix} decreases rapidly with frequency in 150mM buffer which may be attributed to the slow decay of the surface potential resulting in effective increase of the Debye length and hence the capacitive impedance. I_{mix} increases with increasing concentration of Hep-B, which is probably due to the increase in the carrier concentration within graphene and hence the conductivity.

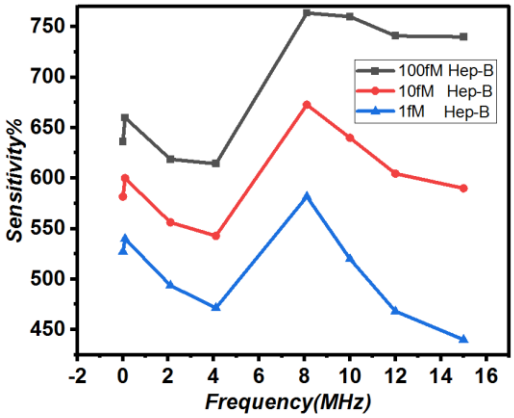


To mitigate the fundamental limit of ionic screening in high ionic strength solution, we operate our ERGO-FET sensor at higher frequencies. Figure 3a, b shows the I_{mix}–V_g characteristics of the ERGO-FET device at f = 100 kHz and 10 MHz, respectively, before and after Hep-B binding. At 100 kHz, change of I_{mix} with different V_g is not prominent and the sensor cannot differentiate the Hep-B binding. But remarkably change of I_{mix} with different V_g has been observed in case of 10 MHz. All the values of I_{mix} suggested ERGO-FET sensor recovered its sensitivity at f > 1 MHz. We observe that at high frequency, the sensor response is enhanced compared to low frequencies and

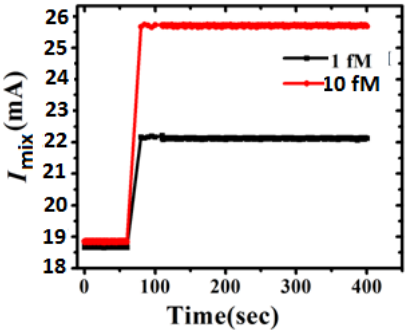
becomes independent of background ionic concentration due to the breakdown of the charge screening EDL. At high frequency, the biomolecules are unable to follow the excitation field and weaken the EDL capacitor. Therefore, with increasing frequency, the EDL screening decreases and ac electric field penetrates deeper into the solution.



We have further plotted the sensitivity Fig 4, calculated as the fractional change in I_{mix} before and after Hep-B attachment. A peak in sensitivity has been observed at a frequency of around 8MHz which corresponds to the optimum interaction of the antigen molecules with the ERGO-FET sensor, overcoming the limitation of small Debye length at 150 mM and the molecules generate a response greater than that at low frequencies. After 8 MHz the sensitivity decreases as the ions are less able to follow the surface asperities.



During the measurements, steady state has been achieved at a certain time, as shown in Fig.5 for ERGO-FET sensors. It is observed that the mean value of I_{mix} starts increasing and after a time of 50 seconds becomes steady at 22.5 mA for 1 fM and 25.5 mA for 10 fM Hep-B concentrations. The sensors have not been re-used, since there might be interferences from previous antigen molecules, if not completely removed.



Conclusions: In this article, we demonstrated electrical biosensing under physiological conditions in high ionic strength solution using high frequency method on smooth ERGO-FET immunosensor, a surface-sensitive two-dimensional material, which effectively regulates the reaction near the surface. The various intrinsic advantages of the sensor have enabled the detection of Hep-B down to 1 fM in 150 mM ionic strength with a high sensitivity.
Hence, ERGO-FET sensor overcame the limitation of Debye screening by the real time biomolecule detection in complex matrices using high frequency.

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