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CNT based high-κ dielectric Ion Sensitive Field Effect Transistor Based Cholesterol Biosensor

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Abstract

Carbon nanotube (CNT) based high-κ dielectric ion sensitive field effect transistor (ISFET) based cholesterol biosensor has been realized using Polyethylenimine (PEI) doped CNT channel and nanocomposite of potassium doped CNT with polypyrrole as sensing membrane.Device has been fabricated using solution method and electrical characterization of the device has been carried out after immobilization of enzyme cholesterol oxidase (ChO_x) in presence of PBS solution.Device has shown a wide dynamic range (0.5 to 25mM), a good sensitivity of 60 mV/ decade,low response time (~1 s), low detection limit (0.23 mM), good stability (7 months) and high selectivity (interference ~1.8 %).

Keywords: CNT, PEI, nanocomposite,cholesterol oxidase, biosensor

Introduction

Biosensors based on the ion-sensitive field effect transistors, so called biologically modified field effect transistors (BioFETs) have been intensively investigated because of their potential advantages such as fast response, low weight, device miniaturization, better fabrication scopes and packaging, high selectivity and sensitivity, high reliability, low cost, on-chip integration.

Enzyme modified field effect transistor (ENFET) is a class of BioFET (1), which is constructed by immobilizing an enzyme on the top of the gate insulator of ISFET. Simplest physics theory behind the working of this device is the exploitation of field effect concept introduced by an electrolyte gate electrode,which is capacitively coupled through a thin dielectric layer. The enzyme reacts with the analyte of interest and generates or consumes protons which changes charge at the gate surface in accordance with site binding theory (2). This change in charge at the gate surface modulates the channel conductance and hence the channel current between the source and the drain of the ISFET. This output current can be related with the concentration of analyte under examination.

Referring to immobilization of enzyme on the top of the insulating surface of ISFET,various advanced materials such as CNT, gold nanoparticles and porous materials have been used for firmed immobilization of the enzyme (3). In general, these materials cannot support enzyme directly, and hence selection of an enzyme-loaded matrix compatible to both the insulating layer and the enzyme has importance in ENFET construction. In the present context, a nanocomposite of N-type carbon nanotube (prepared by doping potassium) with polypyrrole has been used as the enzyme loaded matrix. In general,the compatibility of CNT with enzyme is very high and when it is doped with potassium, it increases free high mobility carriers. Due to this, electron transfer rate increases between the enzymatic reaction site and the oxide layer.Polypyrrole not only provides the support for the enzyme immobilization but also helps in better dispersion of CNT (4). These properties of the nanocomposite can therefore be used to improve the device performance as far as selectivity,

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response time, stability and dynamic range are concerned.

In recent years, CNT based ENFETS (CNT-ENFETs) have attracted great attention because they have been found as ideal biosensor with high selectivity, real-time response and have capability of label free detection (5) -(7). In CNT-ENFETs, the silicon substrate of conventional BioFETs is replaced by semiconducting CNT channel. Polyethylenimine (PEI) doped carbon nanotubes exhibit excellent n-type FET characteristics and has advantage over other types of n-doping techniques because it has the highest density of electron rich amine amongst all the polymers (8), (9). High density electrons of n type CNT results in better transconductance and hence improve the sensitivity of the device.The various intrinsic properties of CNT which are successfully utilized in biosensor development can be obtained elsewhere (10), (11). One of the important properties of CNT that can be exploited to fabricate advanced miniature field effect devices is its compatibility with high-κ dielectric materials (12). Such high-κ dielectric ENFETs have importance in "point-of-care" diagnosis systems and nano-biosensing areas (13), (14).

Referring to cholesterol biomolecule, it is an essential steroid metabolite found in most of the human cells and is carried through blood by lipoproteins (15). Its importance in the design of structure of cell walls, synthesizing bile acids in intestine, production of vitamin D and contribution in making steroid hormones are well known(16). However, elevated level of cholesterol is a biomarker for myocardial infarction, type 2 diabetes, thrombosis, high blood pressure and cardiovascular diseases (17). Therefore, development of a low cost, integrated, disposable and reliable technique for its detection is signiûcant and essential in medical and clinical applications. In this communication, therefore, we have reported the fabrication and characterization of high-κ dielectric CNT based ENFET for detection of cholesterol (CNT-Ch-ENFET).

Material and Methods

Device fabrication and characterization

Device fabrication : Fabrication has been started with cutting of ITO coated glass substrate (purchased from Sigma-Aldrich) using a conventional glass cutter tool(dimension ~ 5 mm × 2 mm). For removal of impurities, ITO coated glass substrate has been washed using a solution of deionized water, ammonium hydroxide and hydrogen peroxide in the ratio 2:1:1. Since, ITO surface is a conducting surface, therefore, to block the conduction from CNT channel to ITO, a layer of ZnO ($\kappa \sim 1.5$) has been deposited on the top of the ITO substrate using solution method, commonly known as electrochemical deposition (ECD) technique. This has been done by using a three electrode electrochemical cell purchased from METROHM (AUTOLAB PGSTAT128N). The details of preparation of solution and its deposition methodology can be obtained in our earlier publication (11).

On the top of ZnO dielectric layer, a thin film of PEI-doped carbon nanotube has been deposited by spin coating technique. Doping of carbon nanotube with 30% polyethylenimine has been done in the presence of EDC-NHS cross linker. The polymer doped carbon nanotubes have been washed with deionized water and filtered using teflon filter paper to remove unbounded particles. The doped CNTs have then been heated at 150^{o}C for 1 hour to remove moisture content. Solution has been prepared by adding 10 mg of PEI doped CNTs in 20 ml of analytical grade acetone. The solution has thenbeen sonicated for 30 minutes using a probe sonicator (The amplitude of the sonicator has been set to 60% with a duty cycle of 50%). For better dispersion, the sonicated solution has been centrifuged using REMI RM 03 at 12000 rpm for 20 minutes. CNT layer has then been deposited using ECD technique. The sample has been heated at 150^{o}C and the process has been repeated until desired thickness of ~ 10 nm has been obtained. On the top of channel region, a thin layer of zirconia has been deposited for serving as the top gate insulator.The cathodic deposition of zirconia layer has been carried out using the same workstation as used for bottom dielectric deposition. To deposit this ZrO_2 layer over channel region, a non-aqueous solution has first been

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prepared by dissolving zirconium tetrachloride in absolute ethanol in the ratio 1:5. The solution has then been sonicated at 20 KHz for 15 min. The deposition process has been carried out by applying constant working electrode potential -1.0 V for about 15-20 minutes. The deposited sample has then been dried using vacuum oven (LABFREEZ) at 250°C for 2 hours. The thickness of this layer is ~10 nm. The source and drain region has been covered with Teflon tape to avoid deposition. Source (S) and drain (D) contacts have been fabricated by physical vapor deposition using metal aluminum. Aluminum (Al) metal has been deposited due to its suitable properties such as low melting point, good adhesion with dielectrics and low resistivity. Then top sensing K/PPy/CNT layer has been deposited on the top of insulating ZrO_2 layer by spin coating technique. The nanocomposite has been prepared by adding 10 ml of 1.0 M KOH to 5 mg of f-CNT. 5 ml of pyrrole solution made in formic acid has then been added to the K/CNT. The solution has been sonicated for 20 minutes using a probe sonicator and kept idle for 12 hours. After the fabrication of the device, 10 µL of ChO_x has been immobilized on the sensing layer by physical adsorption technique (13). Passivation of the device has been done by coating a layer of Polydimethylsiloxane (PDMS). The device has then been dried and kept in refrigeration until used. The schematic of CNT based cholesterol ENFET (biosensor) is shown in Fig 1.

Morphology of all the deposited layershas been investigated using SEM, FESM, TEM results. The layers have been found to be uniform and good adhesion was observed between the layers. The bonding structures of the CNT layer and K/PPy/CNT nanocomposite have been studied using Fourier transform infrared spectroscopy (FTIR) analysis and found the presence of carboxylic acid groups .

Electrical charaterization : The measurement set up for experimentation is shown in Fig 2. Dual tracking power supply has been used for providing desired voltages at drain and gate. The fabricated deviceis immersed in PBS solution of 50 mM concentration and 7.0 pH. Ag/AgCl reference electrode is used for gate biasing.

Prior to being used as ENFET, the device has been biased for electrical characterization. It has been done at pH 7 and changing drain voltage in steps of 0.1 V as shown in Fig 3. From Fig. 3 the biasing points have been fixed at V_{GS} = 0.6 V and V_{DS} = 0.3 V.

Threshold voltage of ENFET can be determined using Extrapolation in linear region

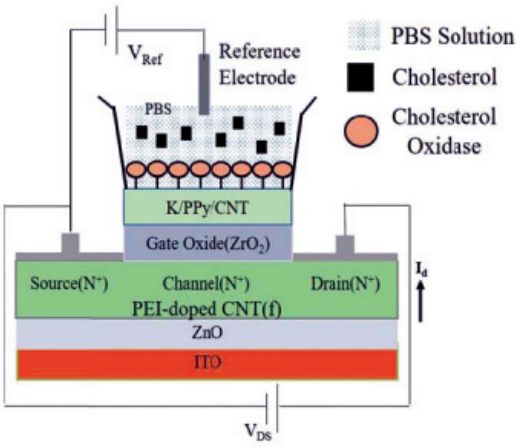

Fig 1. Schematic diagram of cholesterol ENFET

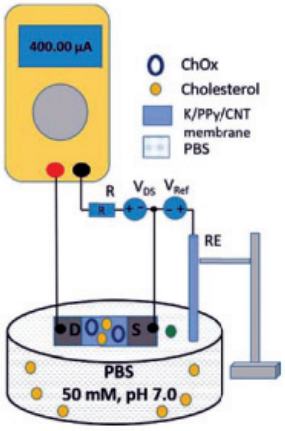

Fig 2. Cholesterol measurement setup

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(ELR) technique (18). Using this technique, the ENFET threshold voltages have been calculated at different cholesterol concentrations from transfer characteristics curve as shown in Fig.4. As far as sensitivity of ENFET device is concerned, it is given by equation 1. This equation implies that the sensitivity is given by the slope of the V_{TH} vs concentration curve as shown in Fig.5.

$$S = \frac{\Delta V_{TH\,(ENFET)}}{\Delta C} \quad (1)$$

Drain characteristics of the cholesterol ENFET have been determined using cholesterol stock solutions of different concentrations.The output characteristics at different concentrations and linearity curve of the device are shown in Fig. 6 and 7 respectively. From Fig.6, it is observed that the dynamic range of the device is (0.5 - 25) mM and from Fig.7, it is observed that the device is linear from 1.8 mM to 19 mM with regression coefficient 0.9943. The current sensitivity has been found to be ~75.17 μA/mM.

Limit of detection is an important parameter in biosensor application. LoD is the least quantity of the analyte which can be detected by the biosensor. It is calculated using eq.2 (19) and found out to be 0.23 mM.

$$LOD = \frac{3 \times \sigma}{S} \quad (2)$$

Where S is the slope between cholesterol concentration and response in terms of interfacial potential and σ is the standard deviation of the response.

Linweaver burk plot shown in Fig. 8 has been used to find the Michaelis-Menten constant (K_m). The value of K_m is found to be 3.4 mM and 2.1mM for ohmic and saturation region of device output characteristics respectively. The value of K_m shows that the device has high affinity towards the analyte.The reproducibility test (Fig. 9) has been performed by characterizing five similar fabricated devices and repeatability test (Fig. 10)has been performedby characterizing the same device for 5

The fabricated device has been compared with other cholesterol biosensors and shown in Table 1.

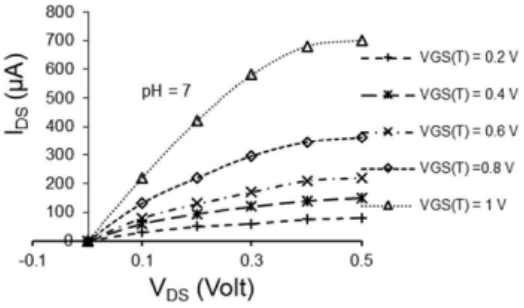

Fig.3. Output characteristics of cholesterol ENFET at pH =7

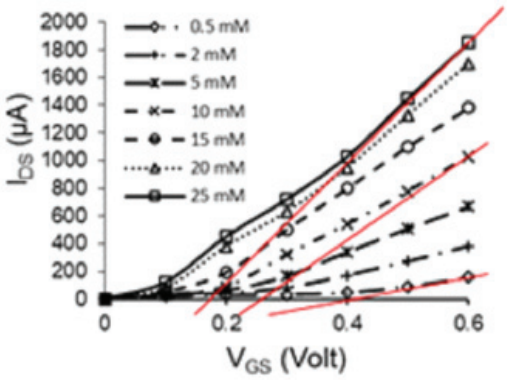

Fig.4. Tranfer characteristics of cholesterol ENFET at various cholesterol concentrations.

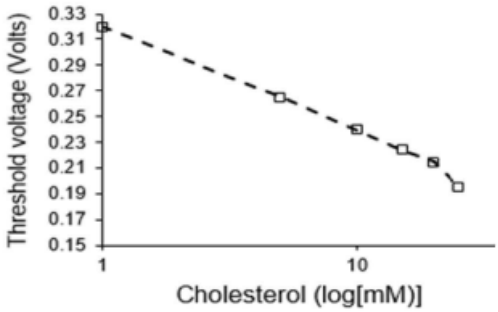

Fig.5. Sensitivity plot

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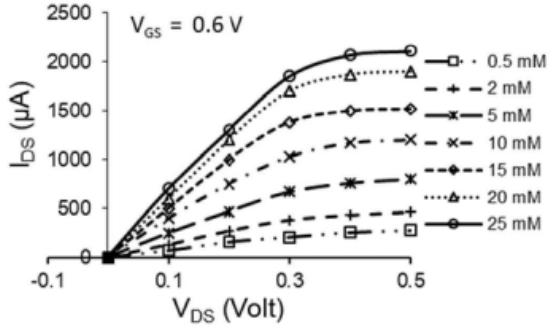

Fig.6. Drain characteristics of cholesterol ENFET

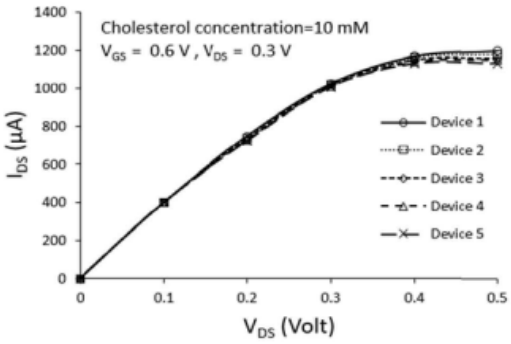

Fig. 9. Reproducibility plot

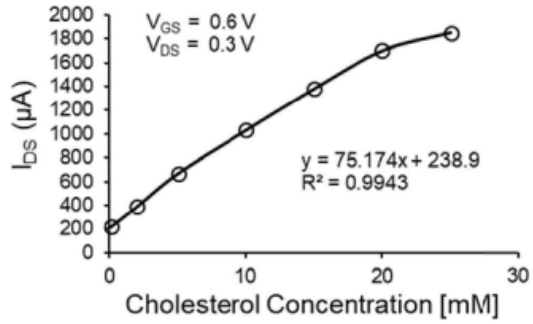

Fig. 7. Transfer characteristics of cholesterol ENFET

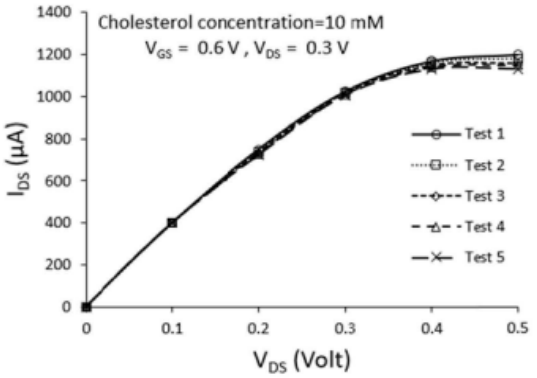

Fig. 10. Repeatability plot

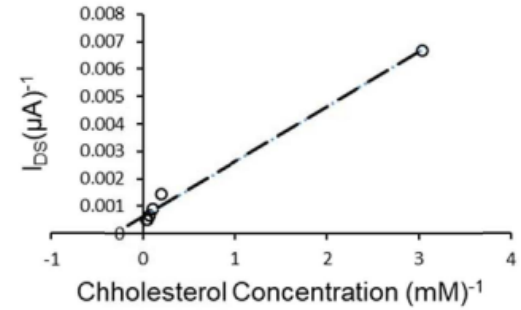

Fig. 8. Linweaver Burk Plot

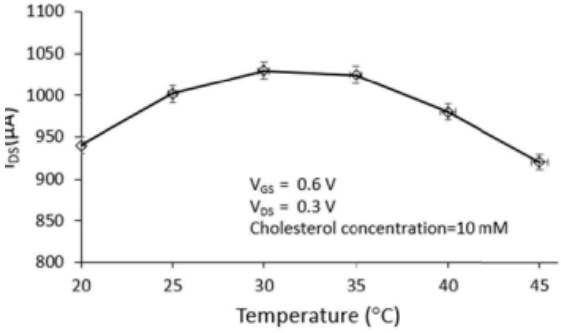

Fig.11. Effect of temperature on drain current

times (once a week). The measurements have been performed under similar conditions.

Temperature dependence of the device has been shown in Fig. 11. In the temperature range of 20 to 40°C, max response is obtained at 30°C. Degradation of enzyme activity is responsible for lowering of drain current at higher temperatures. The plot in Fig.12 shows that the interference of CNT based cholesterol ENFET is negligible with other biomolecules such as glucose, uric acid, urea etc.

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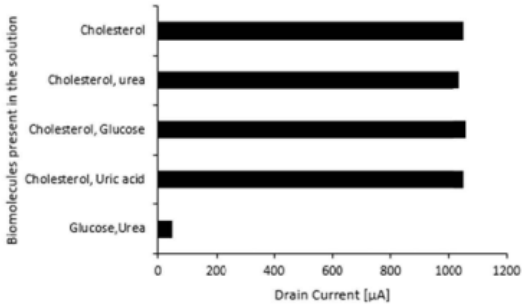

Fig.12. Interference of cholesterolENFET with other molecules

Conclusion

In this work, ENFET with CNT nano composite as sensing layer hasbeen fabricated and characterized for cholesterol detection. Due to the use of K/PPy/CNT nanocomposite, the sensor hasshown high dynamicrange, low detection limit, good stability, goodresponse time and good sensitivity of approximately Nernstian limit. Also the fabricated device has been found to be repeatable and reproduciblewith almost zero interference.

Table 1: Comparison of different cholesterol biosensors

Sensor type	Sensing material	Linearity (mM)	Km(mM)	Sensitivity	Ref.
Solution gated- FET	ZnO	1×10^{-3} -45 and 10μA/mM/cm2	-	-	(20)
Extended gated-FET	Ferrocenyl/ alkanethiol	1.8-12.9	-	43.13 mV/decade	(21)
Potentiometric	ZnO /Ag	1×10^{-3}-10	-	35.2 mV/decade	(22)
Potentiometric	Co3O4	1×10^{-4}- 1	0.039	94.031 mV/decade	(23)
CNT-ENFET	K/PPy/CNT	1.8- 19	2.1	60 mV/decade	This work

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