Fabrication and characterization of junctionless carbon nanotube field effect transistor for cholesterol detection

Md. Abdul Barik and Jiten Ch. Dutta

Citation: Applied Physics Letters **105**, 053509 (2014); doi: 10.1063/1.4892469
View online: http://dx.doi.org/10.1063/1.4892469
View Table of Contents: http://scitation.aip.org/content/aip/journal/apl/105/5?ver=pdfcov
Published by the AIP Publishing

Fabrication and characterization of junctionless carbon nanotube field effect transistor for cholesterol detection

Md. Abdul Barik[a] and Jiten Ch. Dutta
Department of Electronics and Communication Engineering, Tezpur University, Napaam, Tezpur, Assam 784028, India

(Received 6 June 2014; accepted 26 July 2014; published online 7 August 2014)

We have reported fabrication and characterization of polyaniline (PANI)/zinc oxide (ZnO) membrane-based junctionless carbon nanotube field effect transistor deposited on indium tin oxide glass plate for the detection of cholesterol (0.5–22.2 mM). Cholesterol oxidase (ChOx) has been immobilized on the PANI/ZnO membrane by physical adsorption technique. Electrical response has been recorded using digital multimeter (Agilent 3458A) in the presence of phosphate buffer saline of 50 mM, pH 7.0, and 0.9% NaCl contained in a glass pot. The results of response studies for cholesterol reveal linearity as 0.5–16.6 mM and improved sensitivity of 60 mV/decade in good agreement with Nernstian limit ~59.2 mV/decade. The life time of this sensor has been found up to 5 months and response time of 1 s. The limit of detection with regression coefficient (r) ~ 0.998 and Michaelis-Menten constant (K_m) were found to be ~0.25 and 1.4 mM, respectively, indicating high affinity of ChOx to cholesterol. The results obtained in this work show negligible interference with glucose and urea. © *2014 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4892469]

Cholesterol is a waxy steroid metabolite that plays a pivotal role in biomedical and clinical applications. The importance of cholesterol in human metabolism is well known that the defects in cholesterol level leads to complications of heart that may cause hypertension and hypotension.[1] Thus, there is always an increasing demand for the development of new methodologies for simple, rapid, and reliable quantification of cholesterol. Development of highly sensitive, selective, and portable cholesterol biosensor is desirable for identification of hypertension and hypotension in medical diagnosis. Since the development of the first biosensor by Clark in 1962, a variety of concepts and different types of biosensors have been proposed in the past.[2] Among these, the integration of enzyme with an ion sensitive field effect transistor (ISFET) is one of the most attractive approaches. Such a device is known as enzyme modified field effect transistor (EnFET), first proposed by Janata and Moss in 1976 and realized by Caras and Janata in 1980 with penicillin sensitive bioreceptor onto the gate insulator of an ISFET.[3] In the last decade, many such traditional EnFETs have been reported in literatures for the measurement of biomolecules.[4,5] These devices have lot of potential advantages such as small size and weight, fast response, high reliability, low output impedance, on-chip integration, low cost, and mass production.[6–9] Nevertheless, these devices have lot of drawbacks such as high threshold voltage, large internal contact resistance, small ON–OFF current ratio, and complicated fabrication process.[10]

Junctionless field effect transistor (JLFET) is a new concept that can overcome the drawbacks of traditional FET devices. A JLFET has no pn, n^+ n, and p^+ p junction in the source-channel-drain path. It is a uniform resistor having either n-type or p-type material through which mobile charge carriers can be modulated by gate voltage. In the OFF state (at zero gate bias), channel is depleted due to the difference of workfunction between semiconductor and gate electrode. In the ON state (positive gate bias for n-type channel), there is large drain current due to heavily doped charge carriers. The gate modulates the resistance of the heavily doped semiconductor, hence the device behaves like a gated single resistor.[11] JLFETs offer several advantages such as simple fabrication process, low electric field in the ON state, and sharp subthreshold slope.[11,12] But traditional JLFET devices have scaling limitation, viz., if the dimension of the device is reduced by a scale factor λ, then the channel resistance is increased by square of λ.[13] They also suffer from low electrical carrier transport mechanism (diffusion mechanism). These drawbacks can be overcome by carbon nanotube (CNT)-based JLFETs due to the advantages of CNTs such as better scalability (gate length < 20 nm is possible) and high electrical carrier transport property (ballistic transport).[14–17] This Letter reports the fabrication of junctionless carbon nanotube field effect transistor for detection of cholesterol.

Our device fabrication has started with indium tin oxide (ITO) coated glass plate (sheet resistance ~ 15 Ω/cm^2) as a substrate. Prior to being used, ITO glass plate has been cleaned with a solution comprising water, hydrogen peroxide, and ammonium hydroxide (5:2:2) and then rinsed thoroughly with de-ionized water.[18] On the top of the ITO, a layer of zirconium dioxide (ZrO_2) has been deposited using electrochemical deposition (ECD) technique to prevent leakage current from channel to ITO.[18] The deposition technique can be described as follows: 5.0 mg solid zirconium tetrachloride ($ZrCl_4$) has been hydrolyzed in 5 ml water (H_2O) and sonicated for several minutes.[19] Three electrode system consisting of platinum (Pt) wire as counter electrode, silver (Ag)/silver chloride (AgCl) as reference electrode, and ITO as working electrode has been used for deposition and heated at a temperature of 190 °C.[18,19] On the top of the ZrO_2 layer,

[a]Author to whom correspondence should be addressed. Electronic mail: abdulnpl@gmail.com. Telephone: 9707013596.

potassium (K)-doped multiwall CNT (MWCNT) having carbon purity ~ 98% has been deposited using ECD technique.[18] This layer that acts as source (S), drain (D), and channel regions of FET device has been prepared as follows: CNTs (tube length ~ 30 μm and diameter ~ 100–150 nm) have been prepared using catalytic chemical vapor deposition (CVD) technique and functionalized using boiling acid treatment technique before use.[20,21] 5.0 mg CNT has been dispersed in 5 ml acetonitrile and sonicated for 10 min. Since potassium is very reactive with water, that is why, solid potassium hydroxide (KOH) has been dissolved in pure water (5.0 mg/5 ml) using magnetic stirrer. KOH solution forms K^+, OH^-, and releases heat. At the cathode, K^+ ions attach to carbon atoms forming compound bond and behave as charge carrier and thus, electrical conductivity of CNTs can increase.[17,22–25] On the channel region of CNT, a thin layer of ZrO_2 (thickness ~ 10 nm has been measured by gravimetric technique) has been deposited as gate insulator of FET using ECD technique as mentioned earlier. The high κ-dielectric ($ZrO_2 \sim 25$) increases gate oxide capacitance and thus enhances drain current and reduces direct tunneling leakage current.[15] A sensing membrane consisting of polyaniline (PANI) with zinc oxide (ZnO) nanocomposite has been deposited on the top of the gate insulator (ZrO_2) using ECD technique.[18] Nanostructured ZnO has been used due to its unique properties including large surface area, high catalytic efficiency, strong adsorption ability, and chemically stability.[26–31] For preparation of ZnO solution, 10 mg zinc acetate (Zn $(CH_3COO)_2$) has been dissolved in 10 ml distilled water and 2 ml ammonium hydroxide (NH_4OH) has been added and stirred at room temperature for several minutes. Biocompatibility of ZnO has been increased by adding 10 μl PANI (1M) in 10 ml ZnO solution.[18,32–34] For the simplicity of immobilization of biomolecule, the channel length (L) and width (W) have been chosen equal to 1 and 2 mm, respectively. Due to low resistivity, low melting point, and excellent adhesion to dielectrics, ease of deposition and no contamination, aluminum (Al) has been used as contacts for source and drain with CNT. The contact has been made quasi-ohmic using depinning technique, where work function of Al is less than that of CNT (*n*-type). CNT has been heavily doped with potassium that raises the Fermi level towards conduction band. Thus, Fermi level of CNT can match with the Fermi level of aluminium.[35] Polydimethylsilaxane (PDMS) has been coated on the whole FET except channel region for passivation purpose at the time of cholesterol measurement.[36] Fig. 1 shows the complete schematic of JLCNTFET device structure.

Prior to being immobilized Cholesterol oxidase (ChOx), intrinsic voltage gain (A_V) has been calculated from the dc characteristics curves of JLCNTFET and has been found to be ~16 using the relation $A_V = g_m/g_{ds}$, where, g_m is called transconductance ~ 25 000 μS ($=\delta I_{DS}/\delta V_{GS}$ at constant V_{DS}) and $g_{ds} \sim 1600\,\mu$S ($= \delta I_{DS}/\delta V_{DS}$ at constant gate voltage, V_{GS}). The ratio of on-off state drain current (I_{ON}/I_{OFF}) was found to be $\sim 16 \times 10^6$ and sub-threshold swing (SS) ~ 62.5 mV/decade (data and graph are not shown).

ChOx with ~24 U/mg activity and cholesterol were purchased from Sigma Aldrich (USA). ChOx solution (1 mg/ml) has been prepared using phosphate buffer saline (PBS)

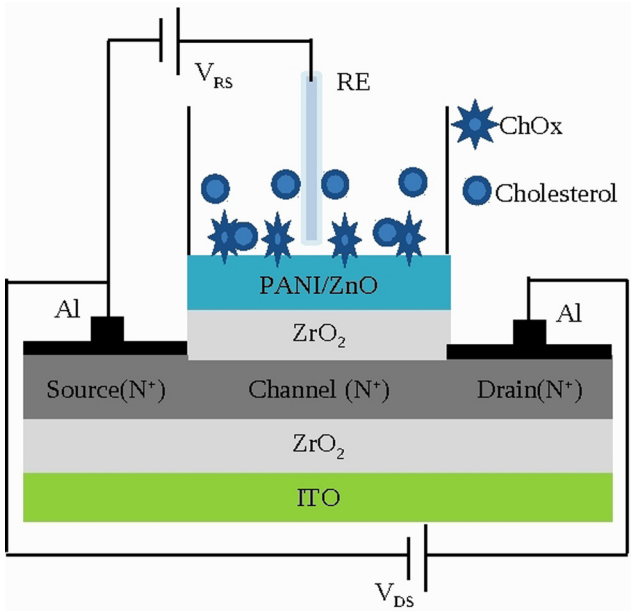

FIG. 1. Schematic of junctionless CNT-based FET for detection of cholesterol.

(50 mM, pH 7.0). Cholesterol stock solution with 25 mM concentration has been prepared using Triton X-100 as surfactant and kept at temperature of 4 °C. PBS having 50 mM, pH 7.0 has been prepared using sodium monophosphate (Na_2HPO_4) and sodium diphosphate (NaH_2PO_4) with 0.9% NaCl and used as mediator. All solutions were prepared using de-ionized water (Milli Q 10 TS) and glassware has been autoclaved prior to use. 1.0 μl of ChOx (1.0 mg/ml, in PB, 50 mM, pH 7.0) has been immobilized onto PANI/ZnO membrane of JLCNTFET using physical adsorption technique, then dried overnight under desiccated conditions and washed with PBS to remove any unbound ChOx and stored at 4 °C when not in use.

For measurement of cholesterol, ChOx immobilized on the device (PANI/ZnO/JLCNTFET) with reference electrode (Ag/AgCl) has been inserted in a glass pot containing 20 ml PBS (50 mM, pH 7.0). Supply voltage (0–0.5 V) in step 0.1 V has been applied between source and drain where positive and negative supply has been connected to drain and source, respectively. Reference electrode voltage (0.5–0.9 V) in step 0.2 V has been applied between reference electrode and source, where positive and negative supplies have been connected to reference electrode and source, respectively. A digital multimeter (Agilent 3458A) has been connected to measure drain current (I_{DS}) as shown in Fig. 2. 10 μl stock solution of cholesterol (0.5–22.2 mM) has been added by micropipette to PBS in the pot each time and corresponding drain current against each cholesterol concentration has been recorded by the digital multimeter. Equation (1) shows the basic principle operation of an EnFET that describes how biocatalytic transformation occurs and stimulated by enzyme that alters pH at the gate surface by generating protons. Here, oxidation of cholesterol with ChOx transforms to cholest-4-en-3-one and H_2O_2 releasing proton (H^+) to the electrolyte solution. These protons affect the potential of the gate interface and consequently affect the potential

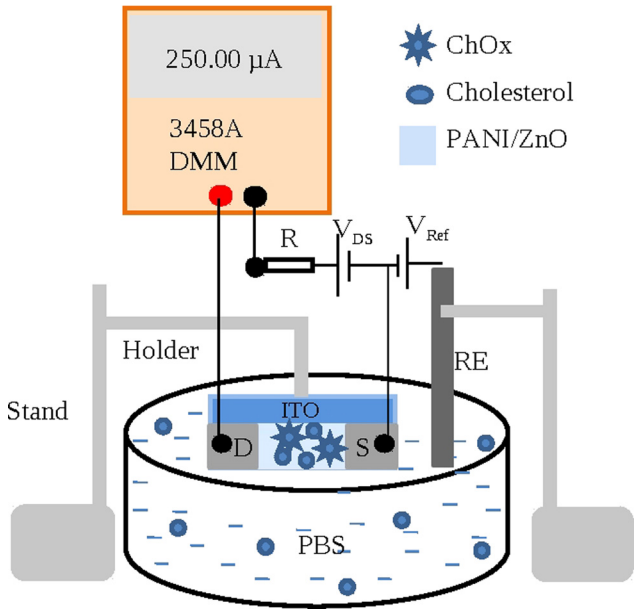

FIG. 2. A system for measurement of electrical response of cholesterol using digital multimeter (Agilent 3458A).

difference between the gate and the source and modulate the channel current[37]

$$\text{Cholesterol} + O_2 \xrightarrow{\text{ChOx}} \text{Cholest-4-en-3-one} + H_2O_2,$$
$$H_2O_2 \xrightarrow{\text{Electrode}} O_2 + 2H^+ + 2e^-. \quad (1)$$

Using setup as shown in Fig. 2, drain current (I_{DS}) has been plotted against drain voltage (V_{DS}) for cholesterol concentration (0.5–22.2 mM) at a fixed reference voltage of 0.6 V at which maximum response obtained as shown in Fig. 3. It has been found that up to drain voltage of 0.3 V, drain current is linear and then saturation occurs just like in characteristics curve of a FET. Therefore, for further experiment, V_{DS} was kept constant at 0.3 V. The different cholesterol concentrations and corresponding drain current has been plotted as shown in Fig. 4. This graph reveals that the device has linearity for cholesterol concentration from 0.5 to 16.6 mM. The

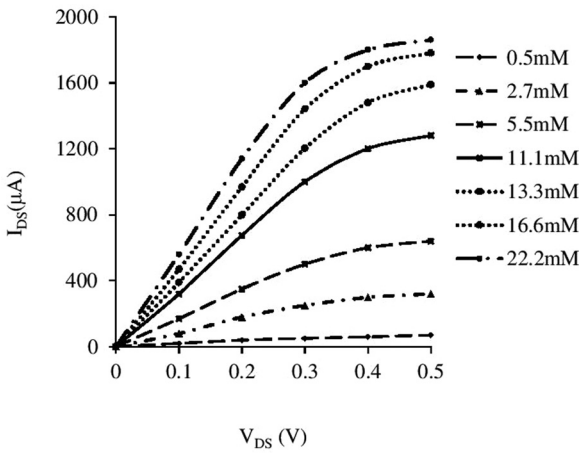

FIG. 3. Electrical responses of different cholesterol concentrations at temperature of 25 °C.

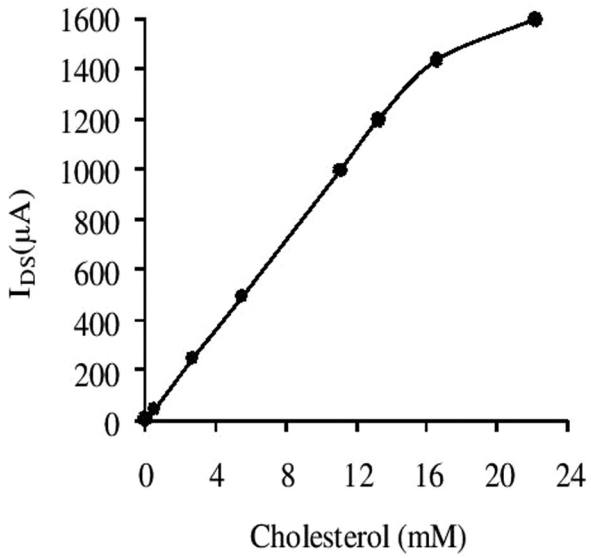

FIG. 4. Variation of electrical response with different cholesterol concentration at drain voltage of 0.3 V and temperature of 25 °C.

Michaelis-Menten constant (K_m) has been calculated from the Linweaver Burk plot (plot of inverse of cholesterol concentration vs. I_{DS}) and was found to be 1.4 mM revealing high affinity immobilization of ChOx with sensing membrane of enzyme JLFET resulting in enhanced biochemical reaction. The interfacial potential (ΔV_{in}) developed at the interface between electrolyte solution and oxide layer of the FET can be calculated using the following equation:[7]

$$\Delta V_{in} = -2.3 \times \frac{RT}{F} \times \log[M], \quad (2)$$

where R is the gas constant and is equal to 8.314 J/K mol, T is the temperature in Kelvin scale at which experiment was performed, F is the Faraday constant and is equal to 9.6×10^4 C/mol, and M is the concentration of electrolyte solution in mol. The interfacial potential developed between solid–liquid phases at room temperature can be calculated using Eq. (2). The sensitivity of an EnFET can be determined by measuring the shift in the interfacial potential of the device. At constant drain current and drain voltage, variation of the interfacial potential was induced by the variation of concentration of electrolyte solution. Thus, sensitivity (S) of an EnFET can be defined mathematically as[38]

$$S = \frac{\Delta V_{in}}{\Delta C_{in}}, \quad (3)$$

where ΔV_{in} is the potential between liquid and solid interface and ΔC_{in} is the pH or concentration of electrolytic solution. Fig. 5 shows the plot of logarithm of cholesterol concentration and interfacial potential at 25 °C. The slope of the graph gives the sensitivity and has been found to 60 mV/decade which is in good agreement with Nernstian response (59.2 mV/decade) at room temperature. The sensitivity also depends on the variation of the interfacial potential of the device due to charge variation on the membrane-solution interface explained by Guoy–Chapman–Stern theory.[37] Limit of detection (LoD) is the lowest quantity of a substance, which

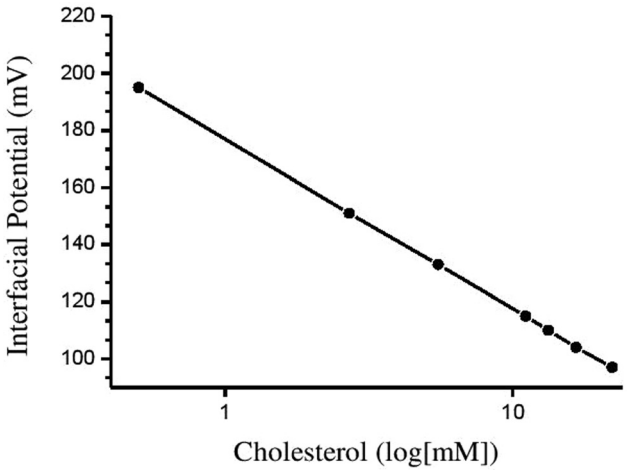

FIG. 5. Effect on interfacial potential due to different cholesterol concentration at drain voltage of 0.3 V and temperature of 25 °C.

can be detected and it is an important parameter for biosensors. Mathematically, limit of detection can be defined as follows and has been found to be 0.2 mM:[39]

$$LoD = \frac{3 \times \sigma}{S}, \tag{4}$$

where σ is called standard deviation and S is the slope of the curve drawn between substrate concentration and their responses. σ has been calculated using the following equation and has been found to7.7 mM:[40]

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}, \tag{5}$$

where x and $\bar{x}$ are the sample value and average value of the samples, respectively, and n is number of samples to be detected. The regression coefficient (r) is a statistical parameter that correlate linearity and mathematically can be calculated as in the following equation[41] and has been found to be ~0.998:

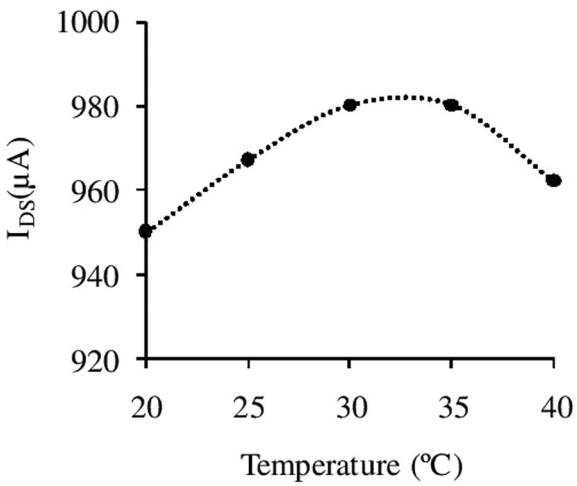

FIG. 6. Effect of temperature on cholesterol responses at drain voltage of 0.3 V and cholesterol concentration of 13.3 mM.

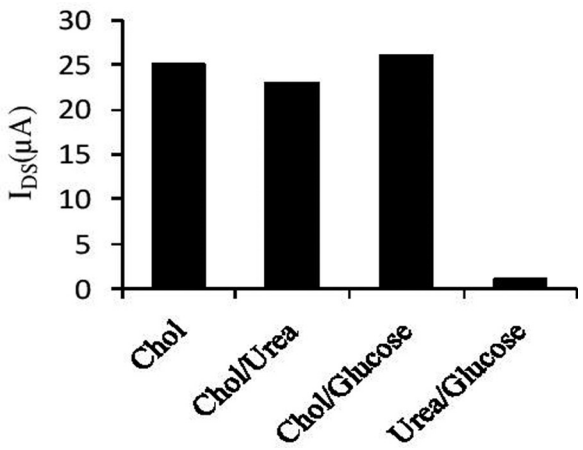

FIG. 7. Effect of interference on cholesterol response with various mixtures at temperature of 25 °C and drain voltage of 0.3 V.

$$r = \frac{\sum \mathrm{dx_i} dy_i}{\sqrt{\sum dx_i^2 \sum dy_i^2}}. \tag{6}$$

The effect of temperature on PANI/ZnO/JLCNTFET has been investigated by measuring drain current using same procedure as mentioned earlier for 13.3 mM of cholesterol solution in PBS (50 mM, pH 7.0) and varying temperature from 20 to 40 °C. The maximum response has been found in the temperature range of 30–35 °C as shown in Fig. 6.

The interference on cholesterol (13.3 mM) solution due to the presence of uric acid (11.1 mM), glucose (13.3 mM), and urea (16.6 mM) has been studied at the same environmental conditions. The results shown in Fig. 7 reveal that there is no significance change in the measured response. The percentage of interference can be calculated using the following equation:[42]

$$\%Interference = \frac{I_{chol} - I_{\mathrm{int}}}{I_{chol}} \times 100, \tag{7}$$

where I_{chol} is the drain current for cholesterol only and I_{int} is the drain current for mixtures. It has been found that average percentage of interference of cholesterol with other solution is ~2%. The experiment has been performed at the same environmental conditions (data not shown) in every week for 5 months and has been found that the device has ~98% activity for cholesterol detection at normal condition. Two cholesterol samples of 5.5 and 16.6 mM were studied using same procedure and condition as mentioned above for about 10 times and only 2% variation of the response has been observed (data not shown). Table I shows comparison of this work with other FET based biosensors reported in literatures.

From the table, it is observed that this device has improved sensitivity, fast response, long stability, and good linearity as compared to other works.

The present study has addressed the fabrication and characterization of CNT based JLFET using electrochemical deposition technique for detection of cholesterol. This integrated device shows improved sensitivity of 60 mV/decade which is in good agreement with Nernstian response of 59.2 mV/decade. This miniaturized device requires minimal instrumentation and can be rapidly fabricated.

TABLE I. Comparison this work with reported literatures for cholesterol biosensor using FET ["-" means no data found].

Sensor type	Sensing materials	Linearity (mM)	Sensitivity	Sensing time (s)	Shelf life (day)	LOD (mM)	K_m (mM)	Reference
Solution gated-FET	ZnO	1×10^{-3}–45	10 μA/mM/cm^2	-	-	5×10^{-5}	-	4
Extended gated-FET	Ferrocenyl/alkanethiol	1.8–12.9	57 mV/decade	-	-	-	-	5
FET	3-aminopropyl triethoxysilane	3×10^{-3}–6.3	48.8 mV/decade	10	-	1×10^{-4}	-	6
FET	Si_3N_4	1×10^{-4}–0.01	43.13 mV/pH	-	-	-	-	38
JLCNT-FET	PANI/ZnO	0.5–16.6	60 mV/decade	1	150	0.25	1.4	This work

Reproducibility, repeatability, and insignificant interference are major factors observed in this work. The efforts are being made to utilize this nano-structured FET based sensor in the field of nano bioelectronics for estimation of other clinical parameters such as glucose, urea, and acetylcholine.

Authors thank the Council of Scientific and Industrial Research, Government of India for the award of Senior Research Fellowship to Md. Abdul Barik (File No. 09/796(0054)/2013-EMR-I). Authors are also thankful to Tezpur University for providing facilities in laboratory.

[1]J. W. Baynes and M. Dominiczak, *Medical Biochemistry*, 2nd ed. (Mosby Ltd., Elsevier, 2005).
[2]L. C. Clark and C. Lyons, Ann. N.Y. Acad. Sci. **102**, 29 (1962).
[3]S. Caras and J. Janata, Anal. Chem. **52**, 1935 (1980).
[4]R. Ahmed, N. Tripathy, and Y. B. Hanh, Biosens. Bioelectron. **45**, 281 (2013).
[5]Y. Ishige, M. Shimoda, and M. Kamahari, Biosens. Bioelectron. **24**, 1096 (2009).
[6]A. B. Kharitonov, M. Zayats, A. Lichtenstian, E. Katz, and I. Willner, Sens. Actuators, B **70**, 222 (2000).
[7]M. Spijkman, E. C. P. Smits, J. F. M. Cillessen, F. Biscarini, P. W. M. Blom, and D. M. de Leeuw, Appl. Phys. Lett. **98**, 043502 (2011).
[8]D. J. Baek, J. P. Duarte, D.-I. Moon, C. H. Kim, J. H. Ahn, and Y. K. Choi, Appl. Phys. Lett. **100**, 213703 (2012).
[9]J. C. Dutta, in *Proceedings of the 2nd National Conference on Computational Intelligence and Signal Processing (CISP)* (2012), p. 185.
[10]T. Tsuchiya, Y. Sato, and M. Tomizawa, IEEE Trans. Electron Devices **45**, 1116 (1998).
[11]J. P. Colinge, C. W. Lee, A. Afzalian, N. D. Akhavan, R. Yan, and I. Farein, Nat. Nanotechnol. **5**(3), 225 (2010).
[12]R. T. Doria, M. A. Pavanello, R. D. Trevisoli, M. de-Souza, C. W. Lee, I. Ferain, N. D. Akhavan, R. Yan, P. Razavi, R. Yu, A. Kranti, and J. P. Colinge, IEEE Trans. Electron Devices **58**, 2511 (2011).
[13]C. M. Osburn and K. R. Bellur, Thin Solid Films **332**, 428 (1998).
[14]R. Sahoo and R. R. Mishro, Int. J. Electron. Eng. Res. **1**, 117 (2009).
[15]A. Javey, H. Kim, M. Brink, Q. Wang, A. Ural, J. Guo, P. Mcintyre, P. Mceuen, M. Lundstrom, and H. J. Dai, Nat. Mater. **1**, 241 (2002).
[16]A. Javey, J. Guo, D. B. Farmer, Q. Wang, D. Wang, R. G. Gordon, M. Lundstrom, and H. Dai, Nano Lett. **4**, 447 (2004).
[17]A. Javey, R. Tu, D. B. Farmer, G. Jonget, R. J. Gordon, and H. Dai, Nano Lett. **5**, 345 (2005).
[18]A. Barik, P. R. Solanki, A. Kaushik, A. Ali, M. K. Pandey, C. G. Kim, and B. D. Malhotra, Nanosci. Nanotechnol. **10**, 6479 (2010).
[19]R. Antti and R. Mikko, J. Mater. Chem. **12**, 1484 (2002).
[20]R. B. Mathur, S. Pande, B. P. Singh, and T. L. Dhami, Polym. Compos. **29**(7), 717–727 (2008).
[21]C. Du and N. Pan, Nanotechnology **17**, 5314 (2006).
[22]R. S. Lee, H. J. Kim, J. E. Fischer, A. Thess, and R. E. Smalley, Nature **388**, 255 (1997).
[23]M. S. Alam, A. Barik, and G. A. Armstrong, in *Proceedings of the IEEE Nanotechnology Material and Devices Conference*, USA, Hawaii, October 16–19 (2012), p. 49.
[24]J. Kong, C. Zhou, E. Yenilmez, and H. Dai, Appl. Phys. Lett. **77**, 3977 (2000).
[25]J. Kong, J. Cao, H. Dai, and E. Anderson, Appl. Phys. Lett. **80**, 73 (2002).
[26]P. R. Solanki, A. Kaushik, A. A. Ansari, and B. D. Malhotra, Appl. Phys. Lett. **94**, 143901 (2009).
[27]R. Khan, A. Kaushik, P. R. Solanki, A. A. Ansari, M. K. Pandey, and B. D. Malhotra, Anal. Chim. Acta **616**, 207 (2008).
[28]A. A. Ansari, P. R. Solanki, and B. D. Malhotra, Appl. Phys. Lett. **92**, 263901 (2008).
[29]E. Topoglidis, Y. Astuti, F. Duriaux, M. Gratzel, and J. R. Durrant, Langmuir **19**, 6894 (2003).
[30]E. Topoglidis, C. J. Compbell, A. E. G. Cass, and J. R. Durrant, Langmuir **17**, 7899 (2001).
[31]A. Ali, A. A. Ansari, A. Kaushik, P. R. Solanki, A. Barik, M. K. Pandey, and B. D. Malhotra, Mater. Lett. **63**, 2473 (2009).
[32]S. I. Yoo, B. H. Sohn, W. C. Zin, and J. C. Jung, Langmuir **20**, 10734 (2004).
[33]B. D. Malhotra, A. Chaubey, and S. P. Singh, Anal. Chim. Acta **578**, 59 (2006).
[34]M. Alam, N. M. Alandis, A. A. Ansari, and M. R. Shaik, J. Nanomater. **2013**, 157810.
[35]Z. Liu, M. Kobayashi, B. C. Paul, Z. Bao, and Y. Nishi, in *IEEE International Electron Devices Meeting* (IEEE, 2009).
[36]B. Zhang, Q. Dong, C. E. Korman, Z. Li, and M. E. Zaghloul, Sci. Rep. **3**, 1098 (2013).
[37]P. Norouzi, F. Faridbod, N. E. Esfahani, B. Larijani, and M. R. Ganjali, Int. J. Electrochem. Sci. **5**, 1008 (2010).
[38]U. Hashim, S. W. Chong, and W. W. Liu, J. Nanomater. **2013**, 542737.
[39]S. Hiroshi and I. Kioshi, J. Magn. Magn. Mater. **321**, 872 (2009).
[40]W. B. George, Am. J. Dis. Child. **136**, 937 (1982).
[41]E. F. Schisterman, K. B. Moysich, L. J. Englandet, K. B. Moysich, L. J. England, and M. Rao, BMC Med. Res. Methodol. **3**, 5 (2003).
[42]C. Dhand, S. K. Arya, M. Datta, and B. D. Malhotra, Anal. Biochem. **383**, 194 (2008).