Label free detection of uric acid using Si nanowire PH sensor

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Abstract. In this study, we successfully apply a silicon nanowire FETs biosensor for detection of uric acid according to pH change. The pH sensing experiments of the silicon nanowire FETs shows that average sensitivity of 42mV/pH. We show that silicon nanowire FETs configured as pH sensors can be used for the quantitative detection of uric acid at concentration as low as 2.4mg/dL. The sensor shows a good linearity (R² = 0.99) and sensitivity (60mV/mM) in the concentration range of 3-8mg/dL, with less than 300 sec response time. These results demonstrate that silicon nanowire FETs based biosensor can potentially be served as the diagnosis tool for general clinical examinations.

1. Introduction

Uric acid is a metabolite of exogenous (taken with food) or endogenous purine bases [1]. Due to the relatively low concentration of uric acid found in human serum (reference range from 3.6 mg / dL to 8.3 mg / dL), it is necessary to use a specific and sensitive method for determination. Reductive and enzymatic are the two main types of methods currently used. Among them, the reduction methods are non-specific and include oxidizing uric acid to allantoin with a phosphotungstate reagent to make the tungstate solution blue. Enzymatic methods are specific and include the catalytic oxidation of uric acid to allantoin with urase and the formation of hydrogen peroxide. [1] It is described in the reactions (1) below [2,3]:

\[
\text{Uric acid} + \text{O}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{uricase}} \text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \xrightarrow{\text{catalase}} 2\text{H}_2\text{O}
\]

(1)

From the enzymatic reaction of uric acid we found that the amount of the product is directly proportional to the concentration of uric acid. Therefore, we can use a pH sensor to detect pH changes related to uric acid concentration.

Since the first introduction of a nanowire field effect transistor (FET) in 2001, it has become a promising technology for ultra-sensitive and label-free diagnostic applications [4,5]. Because nanowires have a high surface / volume ratio, using nanowires instead of planar channels in a field effect device should effectively improve sensitivity.

Silicon nanowire FETs(Si-NW FETs) device configured as solution-phase sensors or ion-sensitive FETs(ISFETs) have been demonstrated as biosensors for small molecules[6], DNA[7], proteins and cellular[8] function in recent years. However, the Debye screening length of a solution with a physiological salt concentration is about 0.7 nm, which effectively neutralizes the molecular charge of the binding ligand beyond this distance. Therefore, in order to increase the Debye length, it is
necessary to measure in low-salt buffer when detecting such surface-bound ligands [9]. So in this work, we devised a method which effects a pH change in a solution to detect uric acid using Silicon nanowire FETs based on Eq. 1. pH calibration, sensitivity and respond time of the sensor are also discussed in this paper.

It is a generally accepted theory [10] that behind the sensitivity of semiconductor insulator surfaces to pH changes and charges in electrolyte solutions is due to the existence of surface states at the oxide terminated silicon surface. The existence of Si-O bonds allows for absorption of a proton from the electrolyte solution and thus formation of neutral silanol groups, which can be further protonated to reach a positively charged state. Depending on the pH values of the electrolyte the result can be positively surface charged (low pH values), neutral, or negatively surface charged (high pH). Fig. 1 depicts the basic principle of the site-dissociation theory silanol groups at the terminating surface of silicon oxide can be either neutral in the form of hydroxyl groups, positive (proton acceptor) or negative (proton donor). The total amount of charge (positive and negative) will determine the dependence of surface potential $\psi$ of a bioFET on pH value in the bulk of the electrolyte, and the value of $d\psi/dpH$ will determine the sensitivity of a bioFET.

According to van Hal, the Eq. 2 gives a general expression for the change of surface potential with the change of pH value at the sensor surface,

$$\frac{\partial \psi_0}{\partial pH_B} = -2.3 \frac{k_B T}{e} \alpha$$  \hspace{1cm} (2)

The parameter $\alpha$ represents the sensitivity parameter which describes the responsivity of the sensor surface. The maximum possible value for the sensitivity of the bioFET is defined as Nernstian response (when $\alpha = 1$) and is approximately 60mV/pH.

Fig. 1. The schematics of the semiconductor-electrolyte interface and the site-dissociation model.

2. Experiments

2.1 Chemicals and Materials.

Uricase (EC 1.7.3.3), uric acid (99% purity) and catalase (EC 1.11.1.6) were obtained from Sigma Company. The activities of uricase and catalase are 4.2 units/mg (one unit of uricase will oxidize 1.0µM uric acid into allantoin at room temperature) and 2440 units/mg, respectively. Ferrocencarboxylic acid (FcA; 99%) was purchased from Aldrich Company. All other reagents are reagent grade and can be used without further purification.

2.2 Si-NW FETs fabrication.

The production of silicon nanobelt FETs is supported by Reed's laboratory. We use a five-step photolithography (PL) process similar to that described in Reference 14. FETs are made of silicon-on-insulator (SOI) wafers with 25 nm active silicon and 145 nm buried oxide layers. The specific steps of the 5-step PL are as follows. First, a nanoribbon mesa having a width of 1 µm and a length of 10 µm is etched using a reactive ion, and then back gate etching is performed. The third step is source and drain implantation, and rapid thermal annealing is performed at 1000 °C to achieve the effect of active
doping. Al then contacts the metallization layer and finally contacts the passivation layer (Shipley S1813).

2.3 Si-NW FET uric acid sensor set-up.

Figure 2 shows a cross-section of a Si-NW FET biosensor structure. A silicon "nanoribbon" device with a solution door is used as the sensing device. Solution gating is performed via Ag/AgCl electrode immersed in a solution to investigate the quality of the sensor. At the top of the chip, a trapezoidal fluid delivery system with inlet and outlet (polytetrafluoroethylene (PTFE) tubing) is installed. Uric acid and uricase solution introduced using inlet tube 1 and 2 respectively.

![Fig.2 Schematic of the Si-NW FET biosensor set-up](image)

2.4 Measurement system.

Devices which were supported were diced into 6 mm x 6 mm dies, packaged into a 28 pin chip holder (Spectrum Semiconductor Materials Inc., CSB02892), and wire bonded (West Bond 747677-E79). Fix custom Tygon® tube mixing chamber (~ 30 - 40μl) on chip with epoxy. When screening equipment, the dry current and wet current-voltage (I-V) characteristics are used. And it uses a custom multiplexing system with a National Instruments data acquisition (DAQ) card, NI PCI-6251 and Agilent 4156 semiconductor parameter analyser. The resulting typical leakage current is about 100fA.

In all the measurements, we keep the measurement interval Ids at 0.5 s, while keeping Vds and Vg constant. Among them, set Vds to 0.1 V, and the value of Vg is determined by the Id-Vg measurement before sensing.

2.5 Chemical processing.

Uricase and uric acid all dissolved in 0.01× PBS (PBS,Sigma), which is diluted by 1×PBS(Sigma). Na2CO3 buffer (pH=10.5, 0.01mM) was used to adjust the pH of the uric acid solution, which ensure uricase solution and uric acid solution have the same pH value (pH value is around 9). And if a change in the reference voltage is observed during this period, the change voltage is caused only by a local pH change. Then we got different concentrations of uric acid, ranging from 2mg / dL to 8mg / dL. In this work, the concentration of uricase is 0.1mg/mL.

When the buffer first injected in the PTFE reservoir, the device would shift for about 1000s and then becomes stable. Only after this processing, detection experiment can be done next. Catalase and FeA were also injected into the reaction chamber to catalyze the H2O2. In this paper, all the experiments were done after the device entering into the stable condition.

3. Results and discussions

3.1 Device calibration.

It is necessary to develop a calibration scheme to compare sensing results across devices to address sensor changes (including surface functionalization). Device solution trans conductance $g_m = (\partial I_{ds} / \partial V_{g,sol})_{V_{ds}}$ was used to normalize the sensor responses in this work. Equation 1 is an
analytical model for determining molecular binding affinity, which is obtained by combining this calibration with the Langmuir isotherm. The device threshold voltage shift $\Delta V_T$ can be expressed as

$$\frac{\Delta I_d}{g_m} = \Delta V_T$$

(3)

Fig. 3 shows a typical static $I_d$-$V_g$ curve after uric acid solution or uric acid solution injected into the PTFE tube. By linear fitting of the I-V curve, it can be seen that the change in $g_m$ to be very small. In our experiment, the working voltage ($V_g$) is -2V. So according to Eq. 3 we can transfer current change to voltage change. Since $g_m$ can be regarded as a constant within our measurement range, we can normalize the sensor response using $\Delta I_d / g_m$. We calculated the transconductance, $g_m$, by linear fitting of $I_d$-$V_g$ curve in fig. 4 and found the change in $g_m$ to be very small between uric acid and uricase solution (<1%). And the result of the transconductance $g_m$ is $-2.04267E-7$. All the results listed below were calculated with this value.

![Graph of Id-Vg characteristic of uric acid and uricase solution](image)

3.2 PH calibration of the sensor.

Before testing uric acid, we first characterized the pH sensitivity of the Si-NW FETs biosensor. As shown in Figure 4, the characteristic device response to pH changes is achieved by completely exchanging the sensing reservoir with buffers of different pH. The operating point for this device is $V_{gs} = $-2V, $V_{ds} = $0.1V. The value of $I_d$ of the p-type Si-NW FETs increases with increasing pH value. Here we used three buffers with pH=4, 7 and 10 respectively. In order to calculate the pH sensitivity of the device, firstly we recorded the $I_d$ values in stable condition of different pH buffers, and then calculate the average of these data, next according to Eq. 3 to get the results. In this work, we known $g_m = -2.04267E-7(A/V)$, so the pH sensitivity of the sensor is 42mV/ pH by calculating the data from Fig.4. Considering that the SiO2 sensitivity factor is ~0.7 at this pH, this is nearly an ideal response.
3.3 Uric acid detection.

Following pH calibration of the device, we used it to detect uric acid. Firstly, uricase solution was injected in the PTFE tube, then different concentrations of uric acid were injected in the tube with the same volume of uricase solution, as shown in Fig. 5. From this figure, we can see the increasing of Ids values with the increased of pH after uric acid reaction according to Eq. 1.

![Fig. 4. pH sensing characteristics of Si-NW FETs](image)

![Fig. 5. Real-time sensor responses of uric acid detection. Each curve represents the measurement of different concentration from the same device.](image)

The response time of the sensor is also showed in figure 6, which defined as the time from the stable condition of uricase solution to the stable condition of uric acid and uricase reaction in this paper. The response time is about 300s with different concentration of uric acid.

In Fig. 6 (a), in this range of 2 mg / dL to 8 mg / dL, the device sensitivity shows a linear dependence on the uric acid concentration. It is worth noting that because the increase in uric acid concentration leads to an increase in the local pH in the solution, the slope of the uric acid calibration curve is positive. According to the calibration curve, the sensitivity of the sensor to uric acid is about 60mV / mM. The lowest concentration of uric acid which the device detected is 2.4mg/dL using this kind of device, shown in Fig.6 (a). As shown in this figure, the sensor displayed a good linearity (R2=0.99). Three sets of device were investigated to access repeatability and uniformity, as shown in Fig. 6(b).
Experiments show that Si-NW FETs are highly sensitive to changes in pH and maintain long-term stability. In addition, they can quantify biomolecules under physiological conditions. The above features make it possible to turn the biosensor into a diagnostic tool for general clinical examination.

4. Conclusion

Using silicon nanowire FETs, we successfully demonstrated a way to detect and quantify uric acid by pH change. This approach overcomes the Debye screening limitation associated with nanowire-FETs sensing. The sensor displayed a good sensitivity (42mV/pH) to pH change, good linearity (R² = 0.99) and a sensitivity for uric acid about 60mV/mM. The lowest concentration of uric acid which the device can be detected is 2.4mg/dL which is lower than the normal range in human body. The response time of the sensor is lower than 300s. It is expected that this sensitive, stable and durable Si-NW FETs based biosensor is promising for applications in general clinical diagnosis.

Fig. 6. (a) Relationship between ΔVds and the concentration of uric acid of the Si-NW FETs sensor (device 1). The solid line is the result of linear fitting. (b) Relationship between ΔVds and the concentration of uric acid using three different sets of nanowires sensors.

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