



# Highly specific and sensitive non-enzymatic determination of uric acid in serum and urine by extended gate field effect transistor sensors



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## ABSTRACT

A potentiometric non-enzymatic sensor using off-chip extended-gate field effect transistor (EGFET) with a ferrocenyl-alkanethiol modified gold electrode is demonstrated for determining the uric acid concentration in human serum and urine. Hexacyanoferrate (II) and (III) ions are used as redox reagent. This potentiometric sensor measures the interface potential on the ferrocene immobilized gold electrode, which is modulated by the redox reaction between uric acid and hexacyanoferrate ions. The device shows a near Nernstian response to uric acid and is highly specific. The interference that comes from glucose, bilirubin, ascorbic acid and hemoglobin is negligible in normal concentration range of these interferents. The sensor also exhibits excellent long term reliability. This extended gate field effect transistor based sensors can be used as a point of care UA testing tool, due to the small size, low cost, and low sample volume consumption.

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## 1. Introduction

Uric acid (UA) is the primary end product of purine metabolism. High concentrations of UA in human body have been linked to many diseases, such as gout, Lesch–Nyhan syndrome, cardiovascular disease, type 2 diabetes, metabolic syndrome and kidney stones (Lakshmi et al., 2011), while lower serum values of uric acid have been associated with multiple sclerosis (Spitsin and Koprowski, 2008). As a result, it is clinically important to monitor the concentration of UA in biological fluids for the early stage warning of these conditions and for the diagnosis of patients. To that end, a simple, reliable and inexpensive detecting system, especially in the form of point of care testing, is highly desirable.

Current *in vitro* quantification of UA concentration usually involves the redox properties of UA. The first approach is by using the UA to reduce the phosphotungstate to tungsten blue in an alkaline solution (pH 9–10), which is measured photometrically (Folin and Macallum, 1912). The method is, however, subject to interferences from drugs and reducing substances other than UA. A second approach, which is the current clinical method of UA analysis, adopts an enzymatic method to specifically detect UA. Uricase is used to catalyze the oxidation of UA by oxygen into allantoin, carbon dioxide and hydrogen peroxide (Ali et al., 2011; Sanders et al., 1980; Zhao et al., 2009).

Besides the redox method, other approaches for UA analysis includes high performance liquid chromatography (HPLC) on reversed phase columns along with detection by either UV absorbance (Sakuma et al., 1987) or mass spectrometry (Lim et al., 1978). These methods involve complex sample and reagent preparation steps, and require bulky and expensive spectroscopic equipment to identify the concentration. These drawbacks make them unsuitable to be used for point of care testing.

Electrochemical techniques for UA detection have attracted much attention due to their merits of fast response, simple testing procedure, cheap instrumentation, along with high selectivity and sensitivity (Xue et al., 2011). So far the electrochemical UA detection is primarily done by an amperometric method (Chen et al., 2005). However, the sensitivity of amperometry depends on the electrode area. It is therefore difficult to decrease the sample volume. Thus, a potentiometric sensor is preferred, since signal intensity is independent of detection volume. In general, electrochemical sensor approaches can be divided into enzymatic and non-enzymatic. The enzymatic approach suffers from an enzyme degradation problem (hard to store for a long time). Since UA can be easily oxidized in aqueous solutions, the non-enzymatic approach is feasible and favorable. However, the interference resulting from ascorbic acid must be minimized (Adams et al., 1976). Recent researches adopt chemical modifications on the electrodes to enhance the selectivity (Raj and Ohsaka, 2003; Toghill et al., 2010; Xue et al., 2011; Zen et al., 1997).

In this study, we report a potentiometric non-enzymatic UA sensor based on an off-chip extended-gate field effect transistor (EGFET) with a ferrocenyl-alkanethiol modified gold electrode.

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The hexacyanoferrate (II) and (III) ions are used as redox reagent. This potentiometric sensor measures the interface potential on the ferrocene immobilized gold electrode, which can be modulated by the redox reaction between UA and hexacyanoferrate ions. The EGFET based sensor has shown high selectivity, sensitivity, reliability and accuracy to UA detection in human serum and urine. Its small size, low cost, low sample volume consumption ( $< 10 \mu\text{L}$ ), and easy operation make this device a potential point of care UA testing tool.

## 2. Materials and methods

### 2.1. Chemicals

The following chemicals and reagents were used in the experiments: ethanol, nitric acid, sodium sulfate, and potassium chloride; potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III); pH standard solution (Brand-Nu Laboratories, USA); 11-(ferrocenyl)undecanethiol (Sigma-Aldrich, USA), uric acid (MP biomedical, USA), human serum from male AB clotted whole blood and sterile-filtered (Sigma-Aldrich, USA), human urine from volunteers; glucose (Acros Organics, No. 410955000), ascorbic acid (Ricca Chemical, No.: RDCA0750-100B1), bilirubin (Acros Organics, No. 230225000), and hemoglobin (Pointe Scientific, No. H7506-STD). All reagent solutions were prepared in the 1X phosphate buffered saline (PBS) solution (Sigma-Aldrich, USA). It should be noted that the artificially prepared uric acid in  $1 \times \text{PBS}$ , if not used immediately, should be stored at  $-20^\circ\text{C}$ . EasyTouch GCU Blood Glucose/Cholesterol/Uric Acid Multi-Function Monitoring System (Bioptik Technology, Taiwan, Type ET-301) was used as the control to determine the accuracy of our procedure.

### 2.2. Device fabrication

The off-chip EGFET UA sensor consists two independent parts (Fig. 1): a disposable front-end sensing chip made of gold electrodes and a reusable back-end FET to detect the interfacial potential on the gold electrode. The front-end gold electrode (80 nm Au on top of 20 nm of an adhesive Cr layer) were manufactured by lithography, metal evaporation, and a lift off process on a 4 in. Si wafer with 3- $\mu\text{m}$ -thick  $\text{SiO}_2$  as an isolating layer. The whole device is protected by another layer of  $\text{SiO}_2$  layer except the sensing area and the bonding pads. The front-end sensing chip was wire-bonded into a ceramic chip carrier. The back-end transistors are

commercially available n-channel MOSFET with zero volt threshold voltage (ALD110800, Advanced Linear Devices). A homemade Ag/AgCl electrode was used as quasi-reference electrode. The modular configuration of a separate front-end sensing chip and a back-end transistor chip has clear advantages in terms of cost and disposability. The front end sensing chip could be produced by screening printing techniques to further reduce the cost per chip.

### 2.3. Functionalization of gold electrodes

The ferrocenylalkanethiol modification of the gold electrode is done as follows. 11-(ferrocenyl)undecanethiol was dissolved in ethanol to form a 1 mM alkanethiol solution. The gold electrode chips were dipped into 1 M nitric acid for 15 s and rinsed with DI water. The washed gold electrode chips were then immersed and kept in the alkanethiol solution at room temperature for 24 h to fully functionalize the gold electrodes. After functionalization, the chips were rinsed with pure ethanol and DI before storing it in the 100 mM sodium sulfate solution at room temperature.

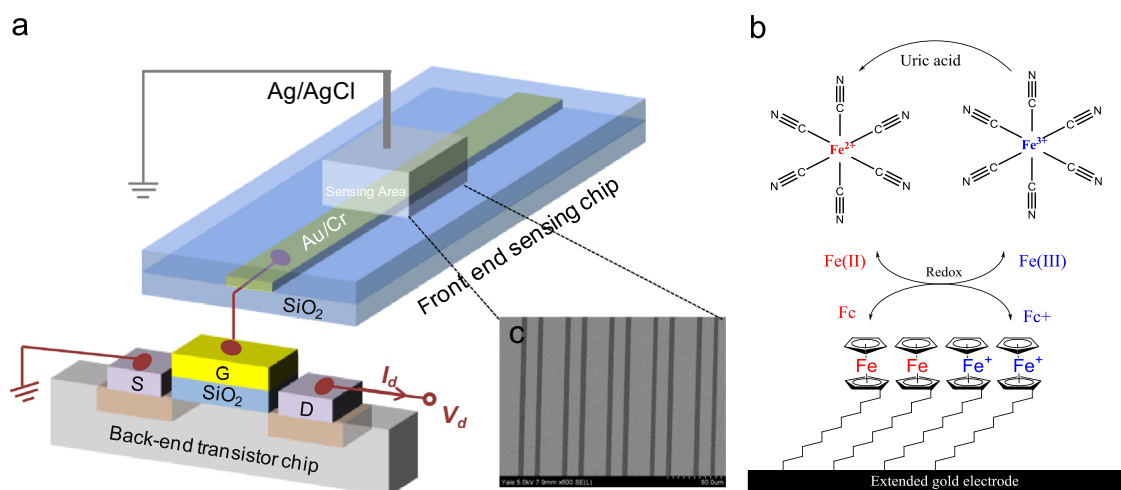
### 2.4. Electrical setup

The front-end sensing chip and the back-end transistors are integrated on a single printed circuit board (PCB), accompanying signal amplification and data acquisition interface for personal computers. The Ag/AgCl quasi-reference electrode is held at a constant potential of 0 V during all tests (Fig. 1). The  $I_d-V_{gs}$  characteristics of off-chip extended gate FETs at a constant  $V_{ds}$  (100 mV) could be tested thoroughly off-line before performing any measurements, serving as a look-up table for converting the measured drain current  $I_d$  back into the interface potential (Guan et al., 2013). All measurements are done at room temperature.

## 3. Results and discussion

### 3.1. Sensing principles

It has been shown that the change in the ratio of redox compound can be detected by the ferrocenyl-alkanethiol modified-FET sensor as the interfacial potential (Ishige et al., 2009). For the ferrocene-modified gold electrode, the interfacial potential ( $E$ ) is determined by the redox state of the ferrocene compounds on



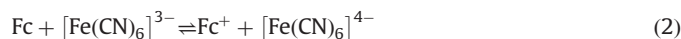
**Fig. 1.** (a) Schematic of the off-chip extended gate field effect transistor sensor configuration. It consists of two independent parts: a disposable front-end sensing chip and a reusable back-end detection transistor. (b) 11-(ferrocenyl)undecanethiol modified gold electrode. The change in the ratio of hexacyanoferrate ions (Fe(II) and Fe(III)) induced through the oxidation of uric acid, can be detected by the ferrocenyl-alkanethiol modified-FET sensor as the interfacial potential, which modulates the drain current ( $I_d$ ) in the FET as shown in (a). (c) Scanning electron microscope (SEM) image of the sensing electrode.

the gold electrode and is given by the Nernstian equation,

$$E = E_0 - \frac{RT}{F} \ln \frac{[\text{Fc}]}{[\text{Fc}^+]} \quad (1)$$

where  $E_0$  is the standard electrode potential,  $R$  is the gas constant,  $T$  is the absolute temperature and  $F$  is the Faraday constant.  $[\text{Fc}]$  and  $[\text{Fc}^+]$  are the ferrocene and ferrocenium ion concentrations, respectively.

Due to their high reactivity with ferrocene compounds on the gold electrodes, hexacyanoferrate ions were widely used as the redox compound, given by the following reaction:



where  $[\text{Fe}(\text{CN})_6]^{3-}$  and  $[\text{Fe}(\text{CN})_6]^{4-}$  is hexacyanoferrate(III) and hexacyanoferrate(II), respectively. At equilibrium, the concentration relationship among the four components in Eq. (2) is described by using thermodynamic equilibrium constant ( $K$ ),

$$\frac{[\text{Fc}]}{[\text{Fc}^+]} = K \frac{[\text{Fe(II)}]}{[\text{Fe(III)}]} \quad (3)$$

where  $[\text{Fe(II)}]$  and  $[\text{Fe(III)}]$  denotes the concentration of  $[\text{Fe}(\text{CN})_6]^{4-}$  and  $[\text{Fe}(\text{CN})_6]^{3-}$ , respectively. By substituting Eq. (3) into Eq. (1), the interfacial potential can be written as

$$E = E_0 - \frac{RT}{F} \ln \frac{[\text{Fe(II)}]}{[\text{Fe(III)}]} \quad (4)$$

Eq. (4) shows that the ratio of hexacyanoferrate (II) concentration to hexacyanoferrate (III) concentration can be determined by measuring the interfacial potential of the ferrocene immobilized gold electrode. In theory, the sensitivity of the interfacial potential should be  $-59 \text{ mV/decade}$  at room temperature.

Morin (1974) described an acid ferric reduction procedure for specifically determining serum uric acid with colorimetric readout, whereby each mole of UA reduces  $N$  moles of ferric ion ( $N \sim 4$ , depending on the pH value and temperature). As a result, by introducing  $V \mu\text{L}$  of UA with a concentration  $[\text{UA}]$  into  $V \mu\text{L}$  of hexacyanoferrate(III) solution with concentration  $[\text{Fe(III)}]_0$ , the concentrations of hexacyanoferrate (III) and hexacyanoferrate (II) after reaction is thus given by

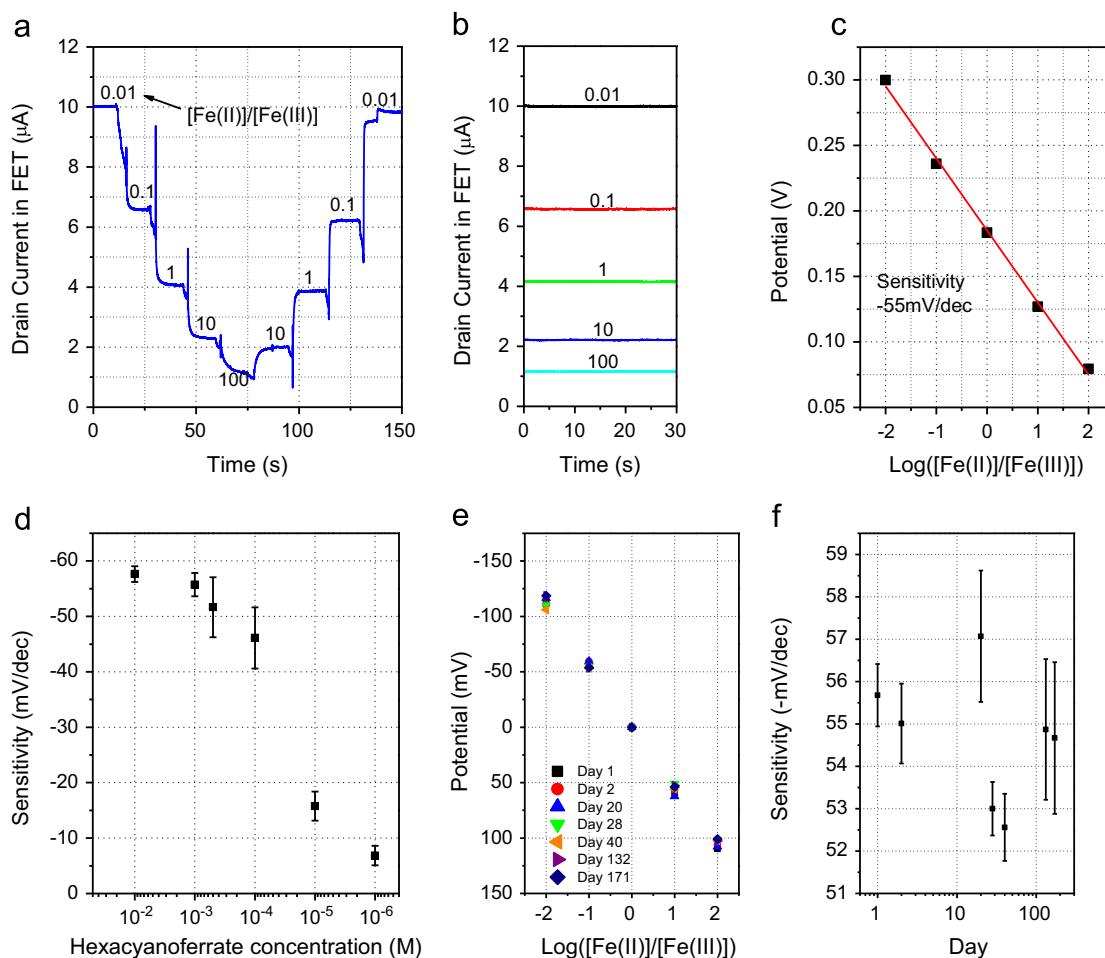
$$[\text{Fe(III)}] = \frac{[\text{Fe(III)}]_0 - N[\text{UA}]}{2}$$

$$[\text{Fe(II)}] = \frac{N[\text{UA}]}{2} \quad (5)$$

Therefore, by combining Eqs. (4) and (5), the interfacial potential can be rewritten as

$$E = E_0 - \frac{RT}{F} \ln \frac{N[\text{UA}]}{[\text{Fe(III)}]_0 - N[\text{UA}]} \quad (6)$$

Under the condition where the initial hexacyanoferrate(III) concentration is much excessive to oxidize the UA, i.e.,  $[\text{Fe(III)}]_0 \gg N[\text{UA}]$ ,



**Fig. 2.** The device response to different  $[\text{Fe(II)}]/[\text{Fe(III)}]$  ratios. (a) Time course of the source-drain current when the device is subject to a continuous set of different  $[\text{Fe(II)}]/[\text{Fe(III)}]$  ratios. The total concentration of the mixed hexacyanoferrate solution is 10 mM, (b) A separate time course for each individual  $[\text{Fe(II)}]/[\text{Fe(III)}]$  ratio at 10 mM concentration, (c) The extracted interface potential as a function of the log ratio of  $[\text{Fe(II)}]/[\text{Fe(III)}]$  for (a) and (b), the slope of which is determined to be  $-55.01 \pm 1.52 \text{ mV/decade}$ , (d) Device sensitivity as a function of the total hexacyanoferrate concentration. (e–f) Long term stability of the device response to different  $[\text{Fe(II)}]/[\text{Fe(III)}]$  values. The interfacial potential values in (e) are normalized at  $[\text{Fe(II)}]/[\text{Fe(III)}] = 1$  to be 0 mV for different days.



UA case in Fig. 3(b) shows a little bit deviation from a linear response due to the fact that  $[\text{Fe(III)}]_0 \gg N[\text{UA}]$  does not strictly hold in this UA concentration range.

To further test the reliability of the device response to UA, we carried out two measurements. The first test is the device to device variation. A total of 6 different devices were tested using a same set of reagents and the device response for each device is almost the same as the data shown in Fig. 3(a and b). The second test is the long term stability of the device response to UA. Fig. 3(c) shows the same device's response to UA of different concentrations for a time period of 140 days. No device response deterioration is observed over this long period of time.

### 3.4. Device response to biological samples

After we determined that the device response to artificially prepared UA is reliable and sensitive, the device was used to determine the UA in biological samples (human serum and urine). Two experiments were carried out to evaluate the performance of the device.

The first is to test the device response to serially diluted biological solutions. Fig. 4(a) shows the end point interfacial potential as a function of dilution ratio when dropping 5  $\mu\text{L}$  serially diluted human serum into 5  $\mu\text{L}$  of 1 mM hexacyanoferrate (III) solutions. The interfacial potential shows a good linearity to the logarithm of dilution ratios. This confirms that the device can work with the biological samples containing complex bio-components.

The second experiment is to determine the UA concentration in biological samples with an assay type setup. The device response to different concentrations of artificially prepared UA solutions was tested to generate a calibration curve (empty squares in Fig. 4(b)). The calibration curve is generated by dropping 5  $\mu\text{L}$  of UA with concentration ranging from 0.1 to 1 mM into 5  $\mu\text{L}$  of 10 mM hexacyanoferrate (III) solutions. The calibration curve can be fitted by

$$E = -0.04709 \log[\text{UA}] + 0.37396 \quad (9)$$

where [UA] is in the unit of mM. Eq. (9) correlates the [UA] with the interfacial potential in this specific test. The end point interfacial potential was also evaluated for biological samples (filled symbols in Fig. 4(b)), the uric acid concentration of which is then derived by using Eq. (9). The determined uric acid concentration in this particular human serum and urine is 3.74 mM and 316  $\mu\text{M}$ . Note that the urine sample is diluted 4 and 8 times so that the UA concentration in the sample is within the calibration range (0.1–1 mM).

After establishing the above procedure to determine the UA concentration in real biological samples, we carried out parallel

experiments with the commercially available method (Biopetik Technology, Taiwan) to verify the accuracy of our procedure. Table 1 summarizes the results from the urine samples of 7 volunteers by the proposed EGFET method and the commercial method. The values of UA in human urine compared favorably to the results obtained from the commercial method. The maximum error between the EGFET method and the commercial method is less than 16%.

### 3.5. Interference

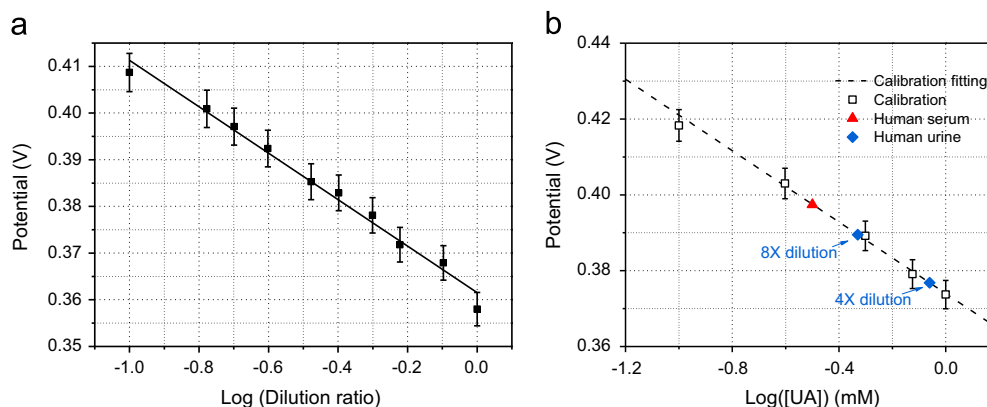
In biological samples such as human serum and urine, common interferences to UA detection are glucose, ascorbic acid, bilirubin, and hemoglobin (Dubois et al., 1989; Morin, 1974). We tested the device response to these interferents at a constant UA concentration (500  $\mu\text{M}$ ) to study the selectivity of the potentiometric uric acid sensor in the present work (Fig. 5).

Fig. 5(a) shows the glucose interference. Upon adding glucose with a concentration from 1 to 20 mM to the UA solution, the potential signal barely changed. The sensitivity to the glucose is around  $-(2.34 \pm 2.16)$  mV/decade. The presence of glucose in the concentration up to 20 mM has no influence on the sensor's response toward UA. Fig. 5(b) shows the bilirubin interference results. Upon adding bilirubin with a concentration from 6.25  $\mu\text{M}$  to 1 mM to the UA solution, the sensitivity to the bilirubin is around  $4.79 \pm 2.39$  mV/decade and it has very little influence on UA detection. Fig. 5(c) shows the ascorbic acid interference. The sensitivity to the ascorbic acid (from 3.9  $\mu\text{M}$  to 1 mM) is around  $-(2.4 \pm 2.21)$  mV/decade. The EGFET sensor thus shows a negligible response to ascorbic acid, which is a major problem in many other electrochemical UA sensors (Chen et al., 2005; Dubois et al., 1989).

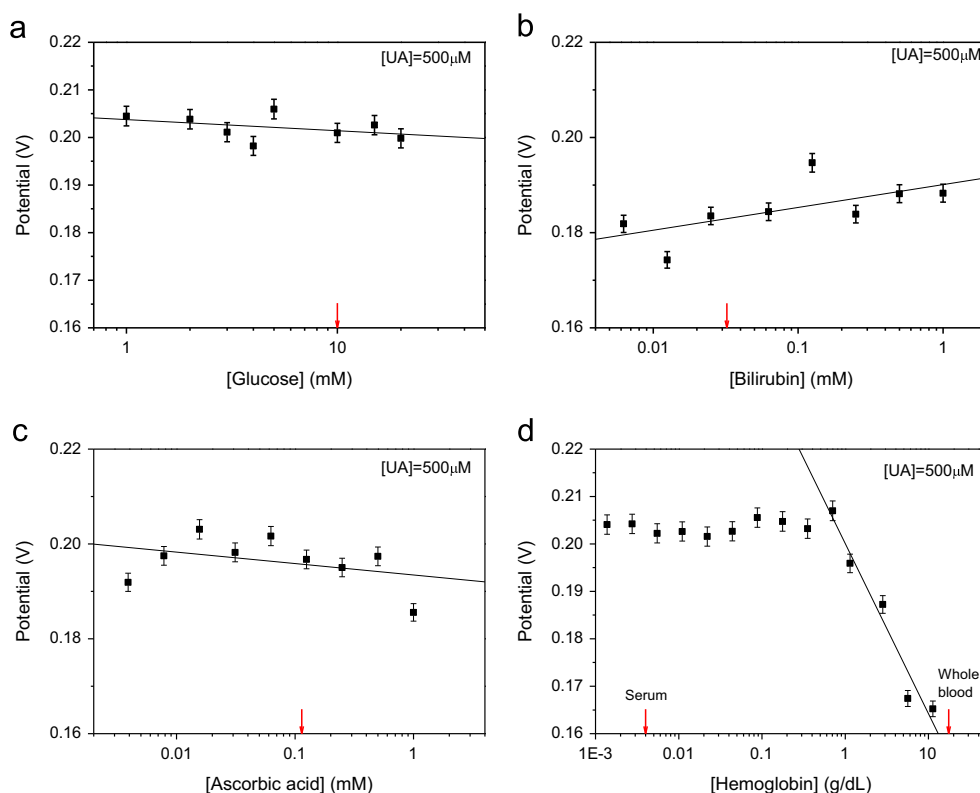
**Table 1**

Comparison between the EGFET method and commercial method using human urine samples.

ID	UA (mM) by commercial method			UA (mM) by EGFET	Error (%)
	1st test	2nd test	Mean		
1	2.94	2.82	$2.88 \pm 0.08$	2.61	−9.44
2	4.90	5.14	$5.02 \pm 0.17$	5.06	0.72
3	4.25	4.25	$4.25 \pm 0.00$	4.56	7.33
4	9.28	0.86	$0.89 \pm 0.04$	0.78	−12.47
5	7.14	0.75	$0.73 \pm 0.03$	0.84	15.39
6	5.80	4.93	$5.36 \pm 0.61$	5.42	0.96
7	5.89	5.35	$5.62 \pm 0.38$	4.88	−13.15



**Fig. 4.** The device response to biological samples. (a) Device response to serially diluted human serum solutions. The sensitivity in this specific device is determined to be  $-49.8 \pm 2.01$  mV/decade. The coefficient of variation of the error bar is 1%. (b) Quantitative detection of uric acid in biological samples. The calibration curve is generated by the known UA concentration (empty squares), which is used to determine the unknown uric acid concentration in the biological samples (filled triangle and diamond). The coefficient of variation of the error bar is 1%.



**Fig. 5.** Interference of (a) glucose, (b) bilirubin, (c) ascorbic acid, and (d) hemoglobin, when dropping interferents with various concentrations into the 500  $\mu\text{M}$  UA solution. The red arrows indicate the reference level of the normal concentration. The slope for each fitted curve is (a)  $-(2.34 \pm 2.16)$  mV/decade, (b)  $4.79 \pm 2.39$  mV/decade, (c)  $-(2.4 \pm 2.21)$  mV/decade, and (d)  $-(35.89 \pm 4.49)$  mV/decade. The coefficient of variation of the error bar is 1%.

We further tested the hemoglobin interference. Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells. As shown in Fig. 5(d), the device response to the hemoglobin remains unchanged upon adding hemoglobin with a concentration from 44 to 706 mg/dL. However, the device shows a sensitivity of around  $-(35.89 \pm 4.49)$  mV/decade to hemoglobin if the concentration is higher than 706 mg/dL. The interference from hemoglobin is mainly due to the fact that the heme group consists of an iron (Fe) ion which is in the ferrous state to support oxygen and other gases' binding and transport. Since the EGFET sensor for UA detection proposed here is based on the  $[\text{Fe(II)}]/[\text{Fe(III)}]$ , additional added ferrous or ferric ions will interfere the results. Therefore, the hemoglobin concentration should be minimized to less than 1 g/dL from a biological sample before using the proposed EGFET uric acid sensor for accurate results. Since the normal hemoglobin concentration is around 1–4 mg/dL in serum and is almost non-existent in urine, the EGFET uric acid can be reliably used for human serum and urine, as shown in Fig. 4(b).

#### 4. Conclusions

We have demonstrated a highly specific, sensitive and reliable non-enzymatic potentiometric method which can be used for the routine determination of uric acid concentration in human serum and urine. By using an extended gate field effect transistor with a ferrocenyl-alkanethiol modified gold electrode, the uric acid can be detected by measuring the interfacial potential. The device shows excellent long term reliability over a period of at least six months. The interference that is associated with glucose, bilirubin, ascorbic acid and hemoglobin are found to be very minimal in the normal concentration range of these interferents. It can be used as

a point of care UA testing tool due to the small size, low cost, and low sample volume consumption ( $< 10 \mu\text{L}$ ).

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