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.

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 ($< 10\,\mu L$), 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 biomedicals, 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 $1 \times$ PBS, if not used immediately, should be stored at $-20\,^{\circ}C$. 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-μm-thick SiO_2 as an isolating layer. The whole device is protected by another layer of 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
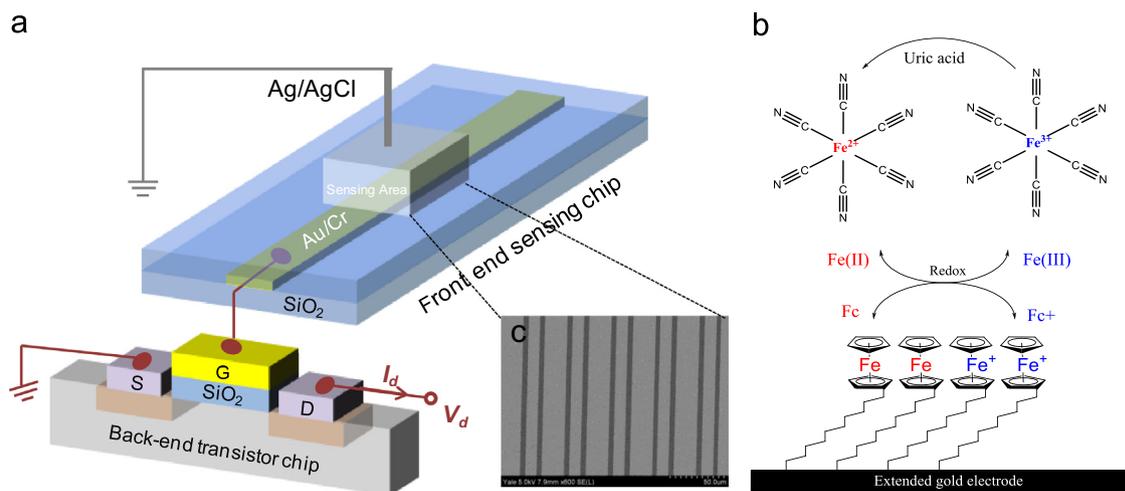
Fig. 1. (a) Schematic of the off-chip extended gate field effect transistor sensor configuration. It consists of two independent parts: a disposable front-end sensing chip and a reusable back-end detection transistor. (b) 11-(ferrocenyl)undecanethiol modified gold electrode. The change in the ratio of 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.