Urea Detection Using Commercial Field Effect Transistors

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We used an easy method to assemble a biosensor using simple and low cost materials. Commercial SnO$_2$:F thin films were used as the basic sensing part of an extended-gate ion-sensitive field effect transistor. When used as a pH sensor, the oxide film showed a sensitivity of $59 \pm 4$ mV$^{-1}$pH$^{-1}$, and linear response in the pH range 2.0 to 10.0. Since urea detection and quantification is important for the control of many pathologies, as proof of concept the samples were further used as platform for urea sensing by immobilizing urease protein onto the surface of the film. For urea sensing, the modified electrodes showed a linear response in the pUrea range of 2.1 to 3.0 (1.00 mM to 7.94 mM, which covers a suitable screening interval for clinical exams) and a sensitivity of $109 \pm 3$ mV/pUrea$^{-1}$. A steady-state was reached after about 60 seconds. Varied pHs and buffer concentrations were also investigated to assess the biosensor behavior toward changes in the environmental conditions. The proposed device could be applied in clinical trials in developing countries as well as in areas hit by natural disasters.

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The ion sensitive field effect transistor (ISFET) is a solid-state device developed in 1970 by Bergveld for physiological measurements. Its fabrication consists in the removal of the gate electrode of a MOS-FET, thus exposing the oxide insulator layer to an electrolyte solution. The device is then liquid-gated by a reference electrode immersed in the same solution. Therefore, the gate-source potential ($V_{GS}$), which modulates the drain-source current ($I_{DS}$), has a contribution due to the reference electrode ($V_{RE}$) and a parcel provided by ions adsorbed at the oxide surface in the inner Helmholtz plane of the Stern-Gouy-Chapman model of the electrical double layer, as given by the site binding model.1,2

The possibility of mass fabrication and miniaturization using thin film technology turned ISFET into a new research field. Van der Spiegel et al., and further Chi et al. developed and upgraded the concept of Extended Gate Field Effect Transistor (EGFET).3,4 In this design, the sensing element was built apart of the FET structure, solving limitations regarding system encapsulation that arises due to the FET close contact with the electrolyte solution. Furthermore, EGFET permits to obtain a new device by only replacing the sensitive film instead of the complete electronic component, which facilitates its fabrication and disposability.5

With the combination of the ISFET concept and the enzyme electrode proposed by Clark and Lyons, Janata and Carus introduced the Enzymatic Field Effect Transistor (EnFET).6,7 Selectivity toward penicillin was conferred to a pH sensitive field effect transistor through penicillinase enzyme immobilization at the gate of an ISFET. Penicillinase catalyzes the hydrolysis of penicillin to penilloic acid, which releases protons thus depressing the pH at the surface of the oxide electrode. This charge variation promoted by the end products of the catalyzed reaction could be used as the main parameter for EnFET operation.7

Urea sensors are one of the most commonly reported in literature because urease is a stable and inexpensive enzyme easily found with high purity in its crystalline form. Hence, it is often used as a proof-of-concept in sensor development.8-10 In the medical field, urea content out of the physiological range could be an indicative of several disorders.11 A healthy person has a physiological blood urea level within the normal range ranging from pUrea 2.1 to 2.6 (2.5 mM to 7.5 mM).12 Others disorders, such as kidney failure, hyperpyrexia, hyperthyroidism, among others disorders.13 A healthy person has a physiological blood urea concentration ranging from pUrea 2.1 to 2.6 (2.5 mM to 7.5 mM).12 Patients with blood serum level higher than 30 mM require hemodialysis treatment or kidney transplantation,13 which reveals the importance in developing new technologies with capability to estimate urea levels in liquids fast and cheap. In addition, urea sensing is also useful in cattle handling,14,15 environment monitoring,16,17 and in food science.18,19

Urea hydrolysis is catalyzed by urease as described by:

\[
\text{(NH}_2\text{)}_2\text{CO + 3H}_2\text{O} \xrightarrow{\text{urease}} \text{CO}_2 + 2\text{NH}_4^+ + 2\text{OH}^- \quad [1]
\]

Herein, we present the development of an urea-EnFET based on EGFET technology using commercial fluorine doped thin oxide (SnO$_2$:F, known as FTO) as the main sensing element. The FTO is one of the less expensive conductor oxides that has been used as alternative to indium tin oxide (ITO), the most used oxide material as electrode in literature.20,21 Device assembly and characterization procedures are presented aside a discussion about environmental conditions such as buffer concentration and pH dependences.

Materials and Methods

Urease from Jack Beans (EC 3.5.1.5, type III, powder, 20990 units/g), glutaraldehyde (25% in aqueous solution) and fluorine-doped tin oxide deposited over glass (FTO, 850 nm-thick, with sheet resistance of $7\Omega$sq. product # 735167) were purchased from Sigma, analytical grade urea (CH$_4$N$_2$O) was purchase from Vetec Química (Brazil). Ethyl acetate and other chemicals such as urea and buffer salts and solutions had analytical grade. Deionized water > 1 M Ω cm (Millipore) was used in all solutions. The FTO samples over glass substrates were sliced in 26 × 10 mm pieces, and connected into the gate terminal of an n-type MOSFET (CD4007UBE from Texas Instruments) using a copper wire and silver epoxy (MG Chemicals). Afterwards, the electric contact area was passivated with epoxy resin leaving an exposed area of 20 × 10 mm (Figure 1a). It should be noted that FTO was chosen over ITO because it is more stable in acid conditions.

Before enzyme immobilization the starting sample was immersed in ethyl acetate for 20 minutes for cleaning purposes. A glutaraldehyde (GA) layer was formed by drop casting 400 μL of GA at 2.5% and left dry 1.5 h in ambient conditions. GA procedure was repeated twice, rinsed with copious amount of deionized water and blow dried with nitrogen. Subsequently, 400 μL of urease solution (25 mg.mL$^{-1}$ in phosphate buffer pH 6.0 at 5 mM) was drop casted on top of the film, and left drying overnight in hood. Weakly bound enzymes were rinsed out through magnetic stirring the samples in the phosphate buffer pH 6 at 5 mM, after which the modified electrode was ready to use (Figure 1b).
The EGFET set-up is shown in Figure 1c. $I_{DS}$ current was measured using a Data Acquisition HP34970, while $V_{DS}$ and $V_{Ref}$ were set with an Agilent E364 Dual Output DC Power Supply controlled through a GPIB interface. Commercial pH buffer solutions with pH 2.0, 4.0, 6.0, 7.0, 8.0, and 10.0 were used to evaluate the pH sensing properties of the bare FTO electrodes. To characterize the modified electrodes, urea concentrations ranging from $pUrea$ 0.5 to 5.0 (10 M to 316 mM) were prepared by diluting the appropriate urea amount in citrate/phosphate buffer. Urea solutions were prepared with pHs 4.9, 5.3, 6.0, 7.0, and 8.0 at 5 mM, 10 mM, 25 mM, and 100 mM, containing 0.1 M NaCl each. The sensing head was immersed in the desired solution and it was water gated by a Hg/Hg$_2$Cl$_2$ reference electrode. Each experiment was performed at least three times, using different samples for each replicate. Each sample was used in a single experiment and discarded, unless otherwise mentioned. All measurements were performed under ambient conditions at 25°C. The morphology of the samples was studied using scanning electron microscopy (SEM). A ZEISS microscope EVO 50 model operating at 20 kV was used. A thin gold coating (≈20 Å) was applied to the samples by means of a Sputter-Coater, Balzers SCD 050.

**Results and Discussion**

Figure 2a shows the source-drain current ($I_{DS}$) as a function of $V_{DS}$, with $V_{GS}$ fixed at 5 V, for the FTO-EGFET sensor. The sensor mechanism could be explained in terms of the site-binding model, which correlates the oxide surface potential ($\Psi_o$) as a function of $H^+$ concentration in the medium through the MOSFET equation:22

$$V_{FB} = V_{Ref} - \Delta\Psi_o + \chi_{sol} - \frac{\Phi_i}{q} - \frac{Q_s + Q_{ox}}{C_{ox}}$$  \hspace{1cm} [2]$$

where $V_{FB}$ is the MOSFET flatband potential, $V_{Ref}$ is the potential applied by the external reference electrode in the medium, $\chi_{sol}$ is a constant potential drop due to oriented water molecule dipoles at the solution/insulator interface, $\Phi_i$ is the work function of the semiconductor, $Q_s$ is the charge at the semiconductor surface, $Q_{ox}$ is the fixed oxide charge in the MOSFET, and $C_{ox}$ is the gate oxide capacitance. The change of $V_{FB}$ (Equation 2) can be indirectly observed in terms of the shifting of threshold voltage,23 which is proportional to the amount of charge adsorbed onto the oxide surface (Figure 2b). Therefore, due to the potential that arises from the adsorbed ions at the FTO surface, for lower pHs of the medium the same arbitrary current can be achieved for smaller $V_{Ref}$ values. An analytical curve was made for a fixed $I_{DS}$ current of 2 mA and the result is shown in the inset of Figure 2b. The resulting linear regression leads to a sensitivity of 59 ± 4 mV.pH$^{-1}$ and R-square of 0.97, in agreement with the maximum theoretical value given by Nernst-equation.26 This result is better than previously published numbers for FTO (50 mV.pH$^{-1}$), and for ITO (43 mV.pH$^{-1}$).27,28

After the FTO pH-response evaluation, new samples were used to immobilize urease. The immobilization procedure was expected to lead to a layer of enzymes at FTO surface, as schematically shown in Figure 1b. GA undergoes auto polymerization forming large domains on FTO surface as observed by SEM (Figure 3a). The GA aldehyde groups present on these structures (which present a porous profile, as seen in Figure 3b) can be used to covalently bind urease through its exposed amine groups of lysine amino acids. We have also verified the presence of crystal structures all over the film (Figure 3c). Figure 2b shows $I_{DS}$ current versus $V_{Ref}$, the applied potential. The change of $V_{FB}$ (Equation 2) can be indirectly observed in terms of the shifting of threshold voltage,23 which is proportional to the amount of charge adsorbed onto the oxide surface (Figure 2b). Therefore, due to the potential that arises from the adsorbed ions at the FTO surface, for lower pHs of the medium the same arbitrary current can be achieved for smaller $V_{Ref}$ values. An analytical curve was made for a fixed $I_{DS}$ current of 2 mA and the result is shown in the inset of Figure 2b. The resulting linear regression leads to a sensitivity of 59 ± 4 mV.pH$^{-1}$ and R-square of 0.97, in agreement with the maximum theoretical value given by Nernst-equation.26 This result is better than previously published numbers for FTO (50 mV.pH$^{-1}$), and for ITO (43 mV.pH$^{-1}$).27,28

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**Figure 1.** a) Photography of assembled FTO sample with effective area of $20 \times 10$ mm$^2$. b) Schematic illustration of urease modified FTO electrode. c) Schematic illustration of EGFET electronic system setup.

**Figure 2.** Response of bare FTO samples to buffer solutions for pH varying from 2.0 to 10.0. a) Drain-Source current ($I_{DS}$) as a function of Drain-Source Voltage ($V_{DS}$) for a fixed $V_{Ref} = 5$ V. b) $I_{DS}$ as a function of $V_{Ref}$ for a fixed $V_{DS} = 5$ V. The inset shows FTO analytic curve calculated for $I_{DS} = 2$ mA. The arrows point to the lowest (pH 2.0) and the highest (pH 10.0) pH used.
sample was rinsed and kept in the same buffer (without urea) for 250 s before the subsequent measurement. This procedure was performed to ensure the enzyme layer had always the same initial tridimensional configuration before each measurement. Signal attenuation strongly occurs during 60 seconds due to the end products of urea catalysis (Equation 1) that decreases the surface potential near the FTO film, until it tends to reach a steady-state. The signal attenuation is more pronounced for larger urea concentrations. Figure 4b shows the analytical curve calculated using the mean value over the last 25 seconds of the data extracted from Figure 4a. Corresponding $V_{GS}$ values were recovered through interpolation of bare transistor $V_{GS}$ curve. The analytical curve leads to a sensitivity of $10^9 \pm 3 \text{ mV \cdot pUrea}^{-1}$, and exhibited linear behavior in the tested urea range from pUrea 2.0 to 3.0 (1.0 to 10.0 mM) with and R-square of 0.99.

For a wider range of urea concentrations, ranging from pUrea 0.5 to pUrea 5.0 (10 μM to 316 mM) and same environmental conditions, the sensor presents a different response (Figure 4c). The sigmoidal curve could be grouped in three distinct regions according to urea concentration, named R1 to R3. A small sensitivity was observed for large urea concentrations (i.e. pUrea smaller than 2.0), evidencing possible urease saturation for R1. Signal changes by only 27% in this region. The biggest signal change of about 67% was observed for urea concentrations between pUrea 2.0 to 3.5, i.e. region R2, which includes the linear range observed in Figure 4b. For lower urea concentrations (pUrea larger than 3.5, region R3) a small shift was observed,
Figure 5. Device response dependence to environmental conditions. a) pH dependence at 10 mM. b) Amplitude response, i.e. difference between highest and lowest urea concentrations, for pHs tested in Figure 5a. c) Buffer concentration dependence for fixed pH 6.0. d) Amplitude response for buffer concentrations tested in Figure 5c.

We performed experiments to assess the sensor behavior at different pHs and buffer concentrations in order to explore the urea sensor performance regarding changes in the environmental conditions. In spite of the loss of mobility expected due to enzyme immobilization, the enzyme activity is also affected by the medium characteristics. Extreme pHs promote changes in enzyme tertiary structure induced by electrostatic forces between charged groups of amino acids chains exposed to the outside,20 which changes its optimal pH of catalysis as well. Figure 5a shows the sensor response as function of urea concentration for several pH values at a fixed buffer concentration of 10 mM. According to the manufacturer, the free urease (EC 3.5.1.5) enzyme has its optimum pH of catalysis around 7.4.31 The enzyme activity was severely suppressed at moderate acid conditions, such as evidenced in the curves for pH 4.9 and 5.3. Moreover, as the reaction products (Eq. 1) alkalize the environment near the sensing film, a high initial pH value suppresses amplitude response, as observed for pH 8.0. pH 6.0 proved to be more suitable for sensor operation in terms of linearity and amplitude response (Figure 5b). Figure 5c shows the sensor response as a function of urea concentration for fixed pH 6.0, at different buffer concentrations. Increasing buffer concentration also increases the buffer capacity, making the environment more resistant against charge variation. Therefore, the curves shift to higher voltage values for increasing buffer concentration. No loss in sensor amplitude response was observed though, which turns the device suitable for operation in varied ambient conditions (Figure 5d).

We have tested the biosensor reproducibility measuring consecutive cycles of five urea concentrations. Each cycle ranges from pUrea 0.5 to pUrea 4.5. Afterwards, the next cycle starts using a solution with pUrea 0.5 again. Figure 6a shows the current ($I_{DS}$) as a function of time for three consecutive cycles. The spikes correspond to sample immersion in urea solutions. The first cycle starts in $t = 0$ and goes to 1250 s, the second cycle from $t = 1250$ s to 2500 s, and the last one from $t = 2500$ s to 3750 s. We observed a small decrease in de-
concentrations were causing loss of activity of the enzyme layer. A notable response over consecutive cycles, causing an experimental error which was proportional to the used urea concentration as observed in Figure 6b. Figure 6b shows the calculated $V_{GS}$ mean values for each urea concentration of the cycles, using the current values from Figure 6a. The results suggest that the pH variations caused by higher urea concentration of the cycles, using the current values from Figure 6b. The signal change caused by the device was turned sensitive to urea through urease immobilization onto the oxide surface. The proposed methodology used simple and inexpensive reagents, easily available in most laboratories in developing countries. Nevertheless, the devices showed acceptable performance to urea quantification in several environmental conditions. Under the optimized conditions of pH 6.0 at 10 mM, a sensitivity of 109 ± 3 mV/pUrea−1 urea concentration was obtained in the linear range of pUrea 2.1 to 3.0 (1.00 to 7.94 mM), which covers the reference range of clinical urea exams. These characteristics highlight the applicability of this new class of sensors for clinical screening exams in high demand areas, such as remote places in development countries or areas hit by natural disaster.

Conclusions

In this work, we demonstrated the usage of commercial FTO thin films as pH sensor based on EGFET technology. As a proof of concept, the device was turned sensitive to urea through urease immobilization onto the oxide surface. The proposed methodology used simple and inexpensive reagents, easily available in most laboratories in developing countries. Nevertheless, the devices showed acceptable performance to urea quantification in several environmental conditions. Under the optimized conditions of pH 6.0 at 10 mM, a sensitivity of 109 ± 3 mV/pUrea−1 urea concentration was obtained in the linear range of pUrea 2.1 to 3.0 (1.00 to 7.94 mM), which covers the reference range of clinical urea exams. These characteristics highlight the applicability of this new class of sensors for clinical screening exams in high demand areas, such as remote places in development countries or areas hit by natural disaster.

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