Detection of saliva-range glucose concentrations using organic thin-film transistors

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We describe the development of a glucose sensor through direct incorporation of an enzyme (glucose oxidase) into the gate of an organic thin film transistor (OTFT). We show that glucose diffusion is the key determinant of the device response time and present a mechanism of glucose sensing in these devices that involves protonic doping of the transistor channel via enzymatic oxidation of glucose. The integrated OTFT sensor is sensitive across 4 decades of glucose concentration; a range that encompasses both the blood and salivary glucose concentration levels. As such, this work acts as a proof-of-concept for low-cost printed biosensors for salivary glucose.

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Development in glucose sensing technology has increased rapidly in recent decades with the heightened incidence of diabetes worldwide. According to a recent report, by the year 2030, the proportion of adult sufferers of diabetes in the world is predicted to be 7.7% of the population (or 438.7×10^6 people) compared with 6.4% in 2010.¹ Currently, people with diabetes need to monitor their blood glucose levels multiple times daily in order to be able to determine appropriate times to eat and to administer medication. Existing sensing equipment used by diabetics requires a small sample of blood to be taken, which can be painful and inconvenient.² As such, there is demand for a more user-friendly, non-invasive approach for measuring the concentration of glucose in blood. In addition, even within the area of existing blood glucose sensors, there is a need for increased sensitivity of the glucose concentration measurement. A recent study of commercial blood glucose sensors by Freckmann *et al.* has shown that many commercial blood glucose sensors suffer from poor accuracy in the high and low blood glucose concentration ranges, which in turn impairs clinical decision making.³

Several reports have already shown that there exists a direct relationship between blood glucose levels and the concentration of glucose in saliva,^{4–7} which is approximately 8–200 μM .⁷ This range of glucose concentration is much lower than the range of concentration for glucose in blood, which is typically 3.3–8.3 mM.⁸ Thus, if current detection sensitivity can be improved, the measurement of saliva glucose levels offers a path towards reliable, non-invasive methods for glucose concentration monitoring in blood.

The detection of glucose has been the subject of much research in the field of organic electronics, and several varieties of sensors have been reported.⁹ Conventional electrochemistry techniques (such as cyclic voltammetry and amperometry) have been employed to determine the concentration of the glucose analyte using the enzyme glucose oxidase (GOX) and these studies have shown promise with very high sensitivities reported.^{10,11} More recently, work has focussed on developing methods for immobilising the GOX in a polymer matrix for use as the electrode in electrochemical cells.^{12–14} Early work by Macaya *et al.* showed that

micromolar sensitivity to glucose could be achieved in organic thin film transistor (OTFT) devices based on the polymer blend, poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS), as the semiconducting channel.¹⁵ This work was further expanded by Kanakamedala *et al.* who showed that these PEDOT:PSS electrochemical devices could be fabricated using an inexpensive and rapid xurography technique.¹⁶ However, all of these previous studies required the GOX to be added to the glucose analyte, which is impractical for a commercial sensor. To date, there have been no reports of an integrated OTFT glucose sensor where the GOX is immobilised within the device structure itself.

In this Letter, we outline the development of a simple OTFT design featuring poly(3-hexylthiophene) (P3HT) as the semiconducting layer, and a gate electrode comprising a film of Nafion (a polymeric membrane material) loaded with a sensing enzyme. This electrode forms the recognition element of the OTFT-based sensor and exhibits sensitivities to glucose in the sub-millimolar concentration range. The work presented here demonstrates a practical self-contained architecture for a printable OTFT-based glucose sensor that is sensitive in the range of concentrations for glucose in saliva, where the enzyme is embedded into the device itself.

Figures 1(a) and 1(b) show the OTFT architectures used for the control OTFT device and the glucose sensing device, respectively. Both architectures are based on source and drain indium tin oxide (ITO) electrodes patterned on a glass substrate.¹⁷ The main difference between the two

FIG. 1. Device configuration of two types of OTFT: (a) a “standard” device and (b) a glucose sensing OTFT featuring the enzyme GOX showing a drop of analyte material. Materials used—A: glass, B: ITO, C: P3HT, D: PVP, E: PEDOT:PSS, F: Nafion:GOX, G: analyte.

architectures is that the PEDOT:PSS gate electrode of the “standard” OTFT is replaced with the Nafion:GOX film. Atomic force microscopy (AFM) images of the devices were used to probe the surface morphology of each layer in the device.¹⁷ Whereas smooth films are obtained for the P3HT ($R_{\text{RMS}} = 0.88$ nm), polyvinylphenol (PVP) ($R_{\text{RMS}} = 1.14$ nm), and Nafion ($R_{\text{RMS}} = 5.20$ nm), the Nafion/GOX films exhibit micron height features on the surface, indicative of aggregates of the GOX enzyme embedded within the Nafion film.

The electrochemistry of the GOX-glucose interaction is well established.¹⁸ When GOX selectively oxidises glucose, gluconolactone, and hydrogen peroxide (H_2O_2) are generated as products of the reaction as described in the following equation:

Subsequently, when the H_2O_2 in solution is oxidised above a threshold voltage (~ 0.7 V versus a standard calomel electrode), it breaks down electrochemically into protons, oxygen, and electrons as described in the following equation:¹⁹

The net result of these two reactions is that a stoichiometric quantity of protons is liberated which is directly proportional to the number of glucose molecules oxidised by the enzyme. In order to confirm the activity of GOX once, it is embedded in a Nafion film, films of Nafion:GOX were deposited onto ITO-coated glass and used as working electrodes in an electrochemical cell containing glucose in the electrolyte. Cyclic voltammetry data using these working electrodes showed that the production of ions from the electrochemical breakdown of H_2O_2 was proportional to the concentration of glucose molecules present in the system as expected.¹⁷

A key advantage of the OTFT architecture used here is its low voltage operation, which makes these devices well-suited for operating with aqueous solutions, since water is known to undergo oxidation at voltages above ~ 1.2 V.²⁰ In order to determine the operating voltage window for oxidation of H_2O_2 in water, the amperometric response of Nafion:GOX films to aqueous H_2O_2 and a deionised (DI) water control was measured. In this configuration, the operating voltage window is defined as the voltage region between the onset of oxidation of the H_2O_2 and the onset of oxidation of water.

As can be seen in Figure 2(a), the onset of oxidation for H_2O_2 occurs at a voltage of ~ 0.75 V, whereas the onset of oxidation for water does not occur until $V \approx 1.5$ V. Therefore, to avoid the oxidation of water contributing to changes in current in the sensing experiments, no electrical potential exceeding 1.5 V should be present in the device, whereas a voltage of at least 0.75 V is sufficient to oxidise (and generate charge carriers from) H_2O_2 .

Figures 2(b) and 2(c) show the output characteristics corresponding to the two types of OTFT devices shown in Figure 1. The device with the PEDOT:PSS gate (Figure 2(b)) shows clearly defined linear and saturation regions as expected for these transistors. Furthermore, this device fully

FIG. 2. (a) Current as a function of voltage between two ITO electrodes connected by a Nafion:GOX film to 10 μl of both 30 mM H_2O_2 solution and DI water, (b) output characteristic of a P3HT/PVP/PEDOT:PSS OTFT, and (c) output characteristic of P3HT/PVP/Nafion:GOX OTFT devices.

turns on at a relatively low voltage of $|V_{\text{GS}}| < 1$ V, which is characteristic of devices of this type.^{21–24} By comparison, the device with a Nafion:GOX layer on top of the PVP dielectric (Figure 2(c)) shows a less-clearly defined saturation region as well as relatively high drain current (I_D) levels when the device is switched off. The deviation from saturation (linearly increasing I_D versus V_{DS} for $V_{\text{DS}} < \sim -1.1$ V) is consistent with a gate electrode offset from the source-drain channel.²² However, for operating source-drain voltages $0 > V_{\text{DS}} > -1.0$ V, the low voltage transistor output characteristics are maintained when the PEDOT:PSS electrode is replaced with Nafion:GOX.

To measure the response of the P3HT/PVP/Nafion:GOX sensor devices to a range of glucose concentrations, a 10 μl drop of analyte was placed on the Nafion:GOX electrode and the drain current (I_D) was monitored as a function of time.¹⁷ V_{DS} and V_{GS} were set to -1 V for the sensing experiments to ensure the OTFT sensor was operating: (i) above the threshold for charge generation from H_2O_2 , (ii) below the

threshold for oxidation of water, and (iii) within the relatively flat saturation region for P3HT/PVP/Nafion:GOX OTFT devices.

Figure 3 shows typical I_D versus time measurements for glucose and H_2O_2 solutions as well as deionised water deposited onto the OTFT sensing devices. There is little change in OTFT device response due to the addition of deionised water, confirming that the applied voltages are below the threshold required to oxidise the solvent. Second, the addition of H_2O_2 results in an immediate and rapid increase in I_D , which reaches a plateau level of $\sim 30 \mu A$, indicating that the applied voltage is above the threshold needed to breakdown H_2O_2 into protons. Finally, the addition of glucose produces a monotonic increase in I_D , which reaches a level ($\sim 25 \mu A$) after 1200 seconds; similar to that of the H_2O_2 case.

We hypothesise that the difference in the temporal response between the H_2O_2 and glucose analytes arises from the slower diffusion and two-stage reaction of glucose with the embedded GOX enzyme compared with the direct diffusion and breakdown of H_2O_2 into protons. To test this hypothesis, we have fitted the 30 mM glucose and H_2O_2 data to a one dimensional solution of Fick's second law of diffusion, $n(x, t) = n_0 \operatorname{erfc}(x/2\sqrt{Dt}) = n_0 \operatorname{erfc}(A/\sqrt{t})$, where n_0 (initial concentration), x (diffusion distance), and D (diffusion constant) are fitting constants. The excellent fit to the glucose and H_2O_2 data indicates that the variation of I_D arises from a diffusion process within the device. From the ratio of the glucose and H_2O_2 data fitting constants ($A_G/A_P = 3.4$), we calculate that the ratio of the effective diffusion constants for glucose and H_2O_2 (D_P^{eff}/D_G^{eff}) is 11.7, suggesting that the effective glucose diffusion is an order of magnitude slower than that of the H_2O_2 .

The operation of these OTFT sensing devices is governed by the generation of charge carriers by the enzymatic reaction and their effect on OTFT current modulation. The current modulation in these hygroscopic OTFT devices is primarily mediated by the diffusion of protons through the dielectric layer.^{21–25} Indeed, we have shown that

FIG. 3. Drain current as a function of time for OTFT sensing devices in the presence of water (small filled red circles), 30 mM glucose (filled red circles), and 30 mM H_2O_2 (large filled red circles). The analyte was added at a time of 300 s in each case. The dashed lines correspond to fits to the 30 mM glucose and 30 mM H_2O_2 data of the solution to the diffusion equation described in Eq. (3).

incorporating a basic polyvinylpyridine (PVPy) dielectric layer leads to proton immobilisation, resulting in low device off-currents.²⁵ Based on this previous work, we propose the following model for current generation in these OTFT glucose sensing devices (Figure 4). As discussed above, glucose diffusion through the PEDOT gate and Nafion to the GOX is slow and likely to be the rate determining step in the mechanism. By contrast, glucose is oxidised rapidly at the GOX to generate gluconolactone and H_2O_2 ,²⁶ which is subsequently rapidly oxidised to protons at the built-in device potential (~ 0.7 V). Moreover, since PVP is a highly hydrated sol-gel material, any enzymatically generated protons will transfer rapidly to the PVP/P3HT interface via the Grotthuss mechanism²⁷ whereupon they dope the P3HT semiconducting layer resulting in the observed I_D changes.

Finally, the OTFT response to a range of glucose concentrations (8 μM –30 mM) was recorded. The dimensionless parameter χ was plotted as a function of glucose concentration where χ is given by

FIG. 4. Model for OTFT glucose sensing mechanism. A: Glucose diffuses to the GOX in the Nafion matrix whereupon H_2O_2 is generated. B: The H_2O_2 is oxidised and protons are generated. C: Protons then diffuse to and dope the P3HT channel.

FIG. 5. Average normalised response ($\log_{10} \chi$) for various glucose concentrations (8 μM , 20 μM , 50 μM , 100 μM , 1 mM, and 30 mM) as a function of $\log_{10} [C_{\text{glucose}}]$. The calibration line is calculated to the data within standard error using least squares fitting. The error of the slope of the calibration line is $\pm 13\%$.

$$\chi = \frac{\int_{t=0}^{t=800} [I_D(t) - I_D(0)] dt}{\int_{t=0}^{t=800} I_D(0) dt}. \quad (3)$$

The parameter χ is defined as the normalised area under the I_D versus time response curve for $0 \leq t \leq 800$ s. By considering the integral of the signal and normalising it in terms of the initial conductivity of the P3HT film, this dimensionless parameter accounts for any variation in the initial conductivity of the P3HT film or rate of change of current. Figure 5 shows a log-log plot of χ as a function of glucose concentration. We observe good correlation between the change in I_D and glucose concentration over 4 decades of glucose concentration. Importantly, this range covers the concentrations observed both in saliva (8–200 μM) and blood (3–8 mM).

In summary, we have shown that GOX can be integrated into low-voltage OTFT devices without loss of enzyme activity or the need for chemical modification of the polymer matrix or enzyme. The integrated sensor shows a response across 4 decades of glucose concentration encompassing both saliva and blood glucose concentration levels. We propose a model for glucose sensing based on protonic doping of the transistor channel, which highlights that the diffusion of glucose is the key determinant of the device response time. This work acts as a proof-of-concept for the integration of functioning biomolecules (such as enzymes) directly into organic electronic devices and demonstrates the feasibility for low-cost printed biosensors for salivary glucose.

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