Micro-Resonator Based Bivariate Detection of Glucose Concentration with Phenylboronic Acid Functionalized Reduced Graphene Oxide

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ABSTRACT We developed a biosensor based on radio frequency micro-resonator for sensitive detection of aqueous glucose solutions. The device incorporates a modified interdigital capacitor-based multilayer resonator realized using integrated passive device technology. In addition, reduced graphene oxide was functionalized by phenylboronic acid for the selective binding of glucose molecules under an electromagnetic field, resulting in a reliable concentration-specific resonance characteristic of the samples. The resonance frequency and bivariate (in terms of magnitude dip and phase reversal of the reflection coefficient) characteristics were found to be linearly related to the glucose concentration, thereby demonstrating the utility of the proposed microwave passive sensor in the detection of glucose with a sensitivity of 0.32 MHz per mg·dL⁻¹. Furthermore, the corresponding variation in the capacitance and the impedance were accessed for each concentration of the glucose solutions.

INDEX TERMS Glucose sensor, integrated passive device, micro-resonator, phenylboronic acid, reduced graphene oxide.

I. INTRODUCTION

The effective management of diabetes requires the continuous monitoring of blood glucose levels. Radio frequency (RF) passive resonators operating in the microwave spectrum have shown enticing capabilities for invasive and noninvasive detection of aqueous glucose concentrations, outperforming the accuracy of broadband coaxial systems [1]–[3]. As compared to electrochemical and optical sensors, RF/microwave sensors offer a robust platform for mediator- and label-free glucose sensing with high sensitivity and reproducibility [4], [5]. Furthermore, these sensors are compact and compatible with the lab-on-a-chip technology to monitor bio-analytes in real time [6].

The spectral response of glucose is mainly governed by the physical diversity in the material electromagnetic properties (e.g., permittivity) that deliver sensitivity in the gigahertz range. Likewise, highly flexible design geometries and proper material selection enable the realization of cost-effective microwave passive sensors. Currently, multilayered conducting resonator-based sensors are being employed given their small chip size and high sensitivity [7], [8]. The study of microfluidic micro and nanodevices enables the contactless detection of aqueous solutions at the microwave regime in biological environments [6], [9].

In this paper, we analyze a high-frequency (in the order of gigahertz) micro-fabricated passive sensor utilizing a modified interdigital capacitor (mIDC) for glucose detection with high sensitivity in the range of microliters. We achieved selective glucose binding by treating the bare sensor chip with phenylboronic acid (PBA) functionalized with reduced graphene oxide (rGO). In fact, PBA acts as a synthetic receptor due to its affinity with diol-containing glucose molecules [10], [11]. Moreover, in fluorescence-based glucose sensors, the effectiveness of PBA functionalization has been shown to increase the sensitivity to glucose-based carbohydrate molecules by the formation of bidentate complex, but not to nonglucose carbohydrate molecules such as galactose [12], [13].

The paper is organized into four sections. Section II provides detailed procedure for the realization of the
proposed design, sample preparation, measurement and surface treatment. Section III summarizes and analyses the bivariate characteristics of the proposed sensor in terms of altered magnitude dip and phase reversal as a function of glucose concentration. This bivariate resonance-based detection methodology is more accurate toward the dielectric characterization of the solutions since the phase reversal property is typically robust towards measurement tolerances [14]. Section IV concludes this paper with main findings, limitations and future works.

II. MATERIALS AND METHODS

A. DESIGN CONCEPT AND REALIZATION

Integrated passive device technology was adopted to realize the resonator sensor in a gallium arsenide (GaAs) substrate with thickness of 400 μm and dielectric constant of 12.85. The 6-inch GaAs wafer was first cleaned and then passivated by 0.2-micron thick silicon nitrate (SiNₓ) dielectric before depositing the seed metal layer of titanium/gold (Ti/Au) in the ratio of 0.2:0.8, respectively. The metal layers comprise copper/gold (Cu/Au) in the ratio of 0.9:0.1, respectively. Details on the fabrication process and materials can be found in our previous work [8], [15].

The fabricated chip was then attached to the printed circuit board using adhesive silver (Ag) epoxy and connected to the copper transmission line by gold wire bonding utilizing thermosonic process. The biosensor chip thus realized had a total area of 1540 × 700 μm² as shown in Figure 1(a). We designed an mIDC structure by connecting two sets of interdigital capacitors through an air-bridge underpass, thus forming three different capacitive interlinks (two from the interdigital structure and one from the air-bridge underpass) as shown in Figure 1(b).

The capacitance of the mIDC is the sum of the conventional interdigital capacitances (C IDC1 and C IDC2) and the air-bridge capacitance (C ab):

\[
C_{\text{mIDC}} = C_{\text{IDC}} = C_{\text{IDC1}} + C_{\text{IDC2}} + C_{\text{ab}}
\]

(1)

whose values can be obtained from [16]

\[
C_{\text{IDC}} = \left[ \frac{\varepsilon_0 \left( \varepsilon_r + \varepsilon_{\text{sub}} \right) K[k]}{2K[k]} + \varepsilon_0 \varepsilon_i \frac{h}{g} \right] (N-1) L, \text{ and}
\]

(2)

\[
C_{\text{ab}} = \varepsilon_0 \varepsilon_i \frac{A}{d},
\]

where \( K[·] \) is the elliptic integral of the first kind with modulus \( k = a/b = 0.6 \) being the ratio between electrode width \( a = 24 \) μm) and distance \( b = 40 \) μm) between the centers of two consecutive electrodes, \( k' = \sqrt{1-k^2} = 0.8 \) is the complementary modulus, \( h = 12 \) μm) is the height of the multilayered conducting line, \( g = 16 \) μm) is the gap between two consecutive electrodes, \( L \) is the length of the electrodes, \( N \) is the total number of electrodes, \( A = 16 \times 16 \) μm²) is the overlapping area of the airbridge, \( d = 1 \) μm) is the height of the air bridge, \( \varepsilon_r = 8.85 \times 10^{-12} \) F/m) is the absolute permittivity, \( \varepsilon_{\text{sub}} = 12.85 \) is the substrate permittivity of the GaAs substrate, and \( \varepsilon_i = 1 \) in free space) is the sample permittivity that depends on the glucose solution. For the bare chip, we calculated the values of \( C_{\text{IDC}} \) and \( C_{\text{ab}} \) to be 0.1489 \( \mu \)F and 0.14 \( \mu \)F, respectively, indicating the dominance of the interdigital capacitance.
matched transmission lines at each port, as shown in Figure 1(c). A subminiature version A (SMA) connector was utilized to connect each port of the device with the analyzer through coaxial cable, whereas an aluminum cube of 2 × 2 × 2 cm³, as shown in Figure 1(d) for grounding and stability. Thus, a bandpass filter characteristic was obtained with resonance frequency $f_o$ of 3.18 GHz and rejection below −30 dB. The measured magnitude and phase response of the fabricated bare sensor chip was shown in Figure 1(e).

**D. SURFACE IMMOBILIZATION AND SAMPLE TESTING**

An rGO solution was injected to the exposed sensor surface followed by the introduction of 2 µL of PBA for glucose immobilization after drying for half an hour. The surface was then cleaned with DI water and 2 µL of each glucose sample was introduced using a micropipette. The chemical binding of rGO and PBA with glucose molecules is illustrated in Figure 2(a).

In Figure 2(b), the peaks in the Raman spectra indicate the existence of graphite at the D band (1372 cm⁻¹) due to distortion and at the G band (1590 cm⁻¹) due to C-C bond stretching. The intensity was reduced by the introduction of PBA due to the formation of cyclic ester [12]. The chemical bonding phenomenon of PBA and rGO before and after the introduction of glucose was reported in [18], where it was demonstrated a decreased shoulder peak due to the binding of PBA and glucose molecules.

The iterative measurement of glucose samples was carried out following the four steps depicted in Figure 2(c). In step (i), the rGO + PBA functionalized chip was connected to the vector network analyzer. The glucose samples (including DI water) were then introduced to the exposed sensing surface, which is the region with mIDC, of the chip in step (ii), and the corresponding resonance characteristics were measured in step (iii). After the RF measurement, the chip was cleaned with DI water in step (iv) and allowed to restore its original RF characteristics before introducing the next sample.

**III. RESULTS AND DISCUSSION**

**A. FREQUENCY-MAGNITUDE RESPONSE**

The frequency dependent resonance characteristics of the proposed sensor as function of the glucose concentration is shown in Figure 3. Resonance frequency $f_o$ and magnitude of the reflection coefficient $|S_{11}|$ were found to be altered with the glucose concentration. Specifically, the resonance frequency shifted from 1.11 to 1.36 GHz with maximum standard error of 0.01, whereas the magnitude of the reflection coefficient decreased from −28.80 to −25.53 dB with maximum standard error of 0.21 for the solution containing from DI water only to 250 mg·dL⁻¹ of glucose, respectively. Three iterative measurements per concentration were performed in the same sensor chip to evaluate the reproducibility and robustness of the resonance characteristics.

**B. SAMPLE PREPARATION**

We extracted rGO by treating graphite oxide prepared using the modified Hummer’s method [17] with deionized (DI) water and acetic acid. The aqueous solution of PBA was prepared by mixing 0.1 mg of PBA powder with 1 mL of ethanol at room temperature. The glucose samples were prepared from 50 to 250 mg·dL⁻¹ to resemble the glucose level in both healthy and diabetic patients. To this end, D-glucose powder was proportionately mixed with DI water to prepare samples with the desired glucose concentration.

**C. MEASUREMENT SETUP**

The RF performance of the fabricated chip was measured using a vector network analyzer (8510C, Agilent Technologies, Santa Clara, CA, USA) calibrated with a two-port network setup and terminated with 50 Ω impedance.
FIGURE 3. Magnitude of the reflection coefficient according to the resonance frequency for different glucose concentrations.

FIGURE 4. Ratios of absolute change in resonance frequency and magnitude of reflection coefficient according to glucose concentration.

The RF/microwave detection of a bio-analyte is governed by the amount of water molecules present in the solution. As the number of water molecules decrease with increasing glucose concentration, the number of polarized ions between two conductors and consequently the capacitance decreases. In turn, the decreased capacitive effect eventually increases the resonance frequency of the passive device as given by the fundamental equation for resonance,

$$f_0 = \sqrt{1/(L_{eq}C_{eq})},$$

where \(L_{eq}\) and \(C_{eq}\) are the equivalent inductance and capacitance, respectively. On the other hand, the decreasing reflection coefficient is due to the loss of applied energy that increases with the glucose concentration.

The resonance characteristics observed in Figure 3 result in the following linear fit relationship for \(f_0\) and \(|S_{11}|\) with glucose concentration \(x\) in mg·dL\(^{-1}\):

$$f_0 \text{ (in GHz)} = 0.0010x + 1.1234, \quad r^2 = 0.9883$$

$$|S_{11}| \text{ (in dB)} = 0.0135x - 29.0408, \quad r^2 = 0.9833 \quad (3)$$

The limit of detection of the proposed sensor computed in terms of the resonance frequency was found to be 7.48 mg·dL\(^{-1}\), which outperforms previously reported air-bridge capacitor [4] and split-ring resonator [8] based sensors. The normalized frequency sensitivity of the proposed sensor given by \(S = (\Delta f_0 / f_0) / \Delta \text{Sample}\), where \(\Delta f_0\) is the average relative change in the resonance frequency and \(\Delta \text{Sample}\) is the difference between the successive sample concentrations, resulting in a sensitivity of 0.32 MHz per mg·dL\(^{-1}\).

The normalized values of the absolute variations in resonance frequency and reflection coefficient are depicted in Figure 4. The value of \(f_0\) and \(|S_{11}|\) were taken from the measured data of the bare sensor chip. The increased value of \(f_0\) and corresponding decrease in \(|S_{11}|\) cause inverse trends in these parameters. In addition, we observed no overlap in the absolute change of these parameters during the iterative measurement, indicating the reproducibility of the proposed sensor.

B. FREQUENCY-PHASE RESPONSE

As shown in Figure 5, a noticeable shift in the phase angle of the reflection coefficient, \(\angle S_{11}\), can be seen in the resonance frequency range. The results are in line with the anomalous dispersion characteristics of the aqueous solutions in the RF.
and microwave frequency range [14]. This double phase reversal method can be utilized to characterize the dielectric properties of the aqueous solutions in the narrowband. The corresponding decrease in the phase angle along with the phase slope indicates a reducing permittivity of the glucose solution [19].

Figure 6 shows the variation in the linear fitting obtained from the phase of the reflection coefficient. The y-intercept and the slope vary from -548.550 to -369.225 and 448.002 to 258.650 for DI water up to 250 mg·dL⁻¹ glucose concentration, respectively.

The linearly decreasing y-intercept and the slope with respect to glucose concentration \( x \) can be obtained respectively as follows

\[
y = 0.72774x - 559.4133, \quad r^2 = 0.9840
\]

\[
y = -0.7425x + 454.1729, \quad r^2 = 0.9838
\]

C. CAPACITANCE AND CORRESPONDING IMPEDANCE

From (5), the dependency of the mIDC impedance for different glucose concentrations can be obtained considering the capacitive effect (for constant values of \( R \)) as summarized in Table 1. We observe a reduced capacitive effect from 1.0739 to 0.6616 pF (and conversely, increased impedance from 133.58 to 176.52 \( \Omega \)) for DI water and glucose solution with 250 mg·dL⁻¹ concentration, respectively. The loaded values of \( C_{\text{mIDC}} \) for the concentration-specific resonance frequencies can be computed by the equations presented in [15, 20] using \( C_{\text{mIDC}} = 0.149 \) pF as unloaded capacitance.

\[
Z = \sqrt{R^2 + \left( \frac{1}{\omega C_{\text{mIDC}}} \right)^2}
\]

TABLE 1. Resonance frequency, capacitance, and impedance of mIDC for different glucose concentrations (mg·dL⁻¹).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DI water</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_0 ) (GHz)</td>
<td>1.1100</td>
<td>1.1833</td>
<td>1.2266</td>
<td>1.2766</td>
<td>1.3170</td>
<td>1.3633</td>
</tr>
<tr>
<td>( C_{\text{mIDC}} ) (pF)</td>
<td>1.0739</td>
<td>0.9270</td>
<td>0.8523</td>
<td>0.7754</td>
<td>0.7196</td>
<td>0.6616</td>
</tr>
<tr>
<td>( Z ) (( \Omega ))</td>
<td>133.58</td>
<td>145.15</td>
<td>152.30</td>
<td>160.84</td>
<td>168.01</td>
<td>176.52</td>
</tr>
</tbody>
</table>

IV. CONCLUSIONS AND FUTURE WORKS

We demonstrated a micro-fabricated passive sensor for the sensitive detection of glucose solutions in DI water. The proposed biosensor effectiveness was verified through experimental evaluation of the RF parameters. We utilized the functionalization of rGO acting as interconnector between two conducting electrodes by PBA for selective binding of glucose molecules. We obtained linear relationships between the bivariate RF parameters and glucose concentration. Thus, the proposed sensor outlines the feasibility of the sensitive RF characterization of glucose solutions in the microwave region.

Future studies should focus on enhancing the detection limit of the sensors in the microwave regime, as well as replacing with the cost-effective miniature active circuit for measurement. The feasibility of the proposed concept can be studied to address the on-demand non-invasive and/or implantable sensor by replacing the toxic GaAs substrate with biocompatible substrate materials such as silicon.

REFERENCES


