Disposable and Flexible Sensor Patch for α-amylase Detection in Human Blood Serum

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Abstract— ***Disposable and flexible sensors are needed in biomedical and healthcare applications because of hygiene requirements. At the same time, they should provide an affordable solution for point-of-care (POC) testing and large-scale deployment. In this view, herein we report flexible polyimide-based disposable sensor patch for the detection of α-amylase in blood serum. The concentration of α-amylase in blood serum is a potential indicator of health issues such as pancreatitis and pancreatic cancer and an affordable solution to detect its concentration could benefit many. Here, the detection is based on thermal Marangoni circulation inside a microfluidic droplet of starch-$FeSO_4$ salt solution, which detects the α-amylase concentration upon addition of blood serum. It was observed that the temperature difference between the droplet substrate and ambient sets a thermal Marangoni and natural convections motion inside the droplet. The performance of the microdroplet-based sensor was best at temperature difference (~18–20°C). The sensor is capable of detecting 20-110 units/liter concentration of α-amylase with ~80% change in the electrical resistance across the microdroplet (at ~40°C substrate temperature), and with a sensitivity of 0.88% (units/liter)$^{-1}$. The response of the sensor was also compared with pathological laboratory results and both were found to be in agreement. The presented sensor has the potential to be used as a POC device for detecting α-amylase in real-time.***

Keywords— Flexible Electronics; Sensor; α-amylase; Disposable Sensor; Point of Care

I. INTRODUCTION

To meet the global health challenges, arising out of demographic changes or pandemic such as COVID, it is important to develop affordable point-of-care (POC) devices [1-4]. The pandemic COVID19 has indicated the immediate need for POC devices for viral infectious diseases including all chronic disorders. Chronic and severe diseases in a person increase the chances of fatality during such spread of infectious diseases, and hence proper and regular monitoring of severe diseases have become critical to reduce the deaths due to comorbidity. In this direction, flexible and wearable devices made from disposable materials could provide efficient and hygienic solutions. Disposable POC testing sensors eliminate the risk of contamination which is vital in biomedical sensing applications. Hence, the development of disposable POC devices is gaining attention day by day [5-9]. The disposable sensors also allow large scale deployment and frequent measurements maintaining the hygienic conditions [10].

In this direction, a variety of biosensors have been developed in recent years to detect biomarkers from body fluids like tears, sweat, urine, etc. [11, 12]. Some of these biomarkers are also capable of early diagnosis of various diseases. For example, the normal range of α-amylase endo-1,4-α-d-glucan glucanohydrolase, EC 3.2.1.1 in healthy human serum is about 25 – 85 U/L (units/liter) [13]. An increase in the concentration of α-amylase value could indicate the onset of diseases such as acute pancreatitis, salivary gland infection, pancreatic cancer, gastroenteritis and bile duct blockage, among many others. Similarly, a reduced level compared to the mentioned concentration could also indicate kidney malfunction, and toxaemia in pregnancy [13], among others. Regular measurement of α-amylase in blood serum could help prevent several diseases.

The existing processes available for the detection of α-amylase in blood serum involve spectroscopy [14-16], fluorometry [17], colorimetry [18], electrochemical methods [19], weight-based detection [20], immunological methods [21], and electromagnetic sensing [22]. Among these, the spectrometry is considered to be the best method, even if it is an expensive process [23]. Further, the operation and analysis of spectroscopic results require skilled personnel. Given that the α-amylase is linked with several diseases, an affordable and disposable sensor and POC device could lead to an attractive healthcare solution. Further, this could enable self-health management, which needs flexible and wearable devices [11, 24-27]. The available solutions are not suitable for the said purpose [28].

In this paper, we discuss a simple detection technique based on a microdroplet and a disposable sensing patch. The sensor works based on thermal Marangoni and natural convection along with starch-amylase reaction [29]. The convection inside helps the reaction to take place homogeneously. A microdroplet of $FeSO_4$ salt solution was used as an active material to detect α-amylase in blood serum. The response of the sensor is in good agreement t with pathological laboratory results. The paper is organised as follows: The materials and methods used in this work are described in Section II. This is followed by discussion related on key results in Section III, and the summary if key findings in Section IV.

II. MATERIALS AND METHODS

A. Materials

The Iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$), starch (potato starch) and the enzyme porcine pancreatic amylase were procured from Sigma-Aldrich and employed in the experiments without further purification. Polyimide flexible

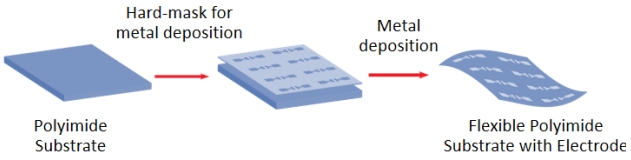

Fig. 1. Schematic illustration of the fabrication steps.

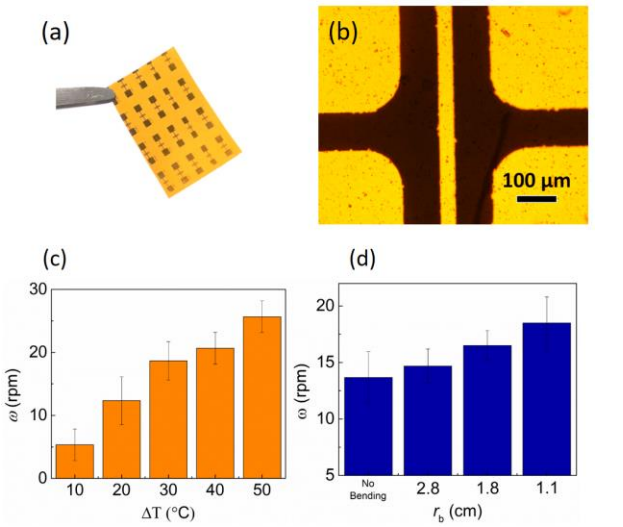

Fig. 2. (a) Optical image of the fabricated flexible sensor patch; (b) microscopic image of the electrodes; (c) rotational speed (ω) with temperature difference (ΔT); (d) the change in rotational speed with bending radius (r_b).

sheet was employed as the substrate for fabrication of the sensors. The Milli-Q grade water was used for cleaning and preparing the solutions.

B. Fabrication of Flexible Sensor Electrodes

The flexible sensor electrodes were fabricated using metal deposition on a polyimide substrate. The fabrication steps of the flexible sensor electrodes are shown in Fig. 1. The fabrication was performed using a hard mask and thermal deposition. In this process, the 50 nm thick Pt was deposited on a 10 nm Au layer to fabricate conductive electrodes having a separation gap of ~40 µm on the flexible polyimide substrate [29]. The purpose of the Au layer deposition was to enhance the adhesion between the polyimide substrate and electrodes. The flexible substrate and the microscopic images of the electrodes are shown in Fig. 2(a) and 2(b), respectively.

C. Electrical Characterization

In order to characterize the sensor, the fabricated electrodes were initially connected to the flexible conductive wire using silver paste. The flexible wires were then connected to a digital multimeter (MASTECH M92A(H)) to record the change in conductance of the microdroplet-based sensor. Thereafter, 1M $FeSO_4$ and 10% (w/v) aqueous starch solution were prepared separately by dissolving salt and starch in appropriate amounts to DI water. The purpose of $FeSO_4$ was to make the microdroplet conductive. The mentioned solutions were then mixed in equal volumes (1:1, v/v) to obtain the 'mixture I'. The substrate integrated with electrodes was then placed on a temperature-controllable hotplate so that the temperature based studies can be performed.

III. Results and Discussion

A. Effect of Temperature on Droplet Rotation

The experiments were performed on the fabricated flexible polyimide sensor with a droplet of $FeSO_4$ solution. The polyimide substrate was selected due to its suitable thermal conductivity (~1.2 W/mK) and temperature sustainability. Apart from this, polyimide has suitable surface energy for this application. Fig. 2(a) shows an optical image of the fabricated flexible sensor electrodes. Fig. 2(b) shows the optical microscopic image of the electrode.

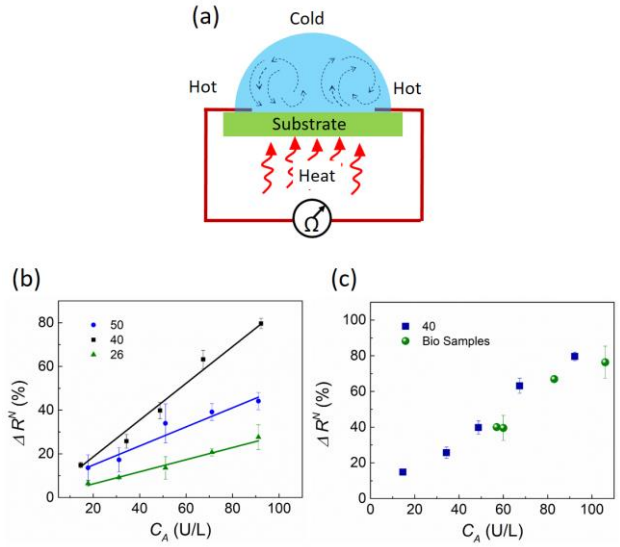

Fig. 3. (a) Schematic illustration of the experimental set-up; (b) the response of sensor (ΔR^N) with different concentration of α-amylase (C_A) and temperatures; (c) the response of sensor (ΔR^N) at 40°C (blue squares) along with the responses for blood serum i.e. biosamples (green spheres).

A series of experiments were conducted for different temperatures to evaluate the response of the fabricated sensor. For that purpose, the sensor with a droplet was placed on a hotplate having a digital temperature controller. The temperature was then increased from 25°C to 75°C and the rotation inside the droplet was recorded using a digital camera. A few small graphite particles (~200µm) were introduced inside the droplet to record the rotation. The rotational speed (ω) inside the droplet with temperature difference (ΔT) between the substrate and ambient is illustrated in Fig. 2(c). It was observed that the rotation increases with the increase of temperature.

The effect of the bending of the substrate was also experimentally investigated. For that purpose, the sensor was placed over different surfaces having bending radii of 1.1, 1.8, and 2.8 cm. The temperature was then monitored using an IR temperature sensing device (HTC). It was observed that the rotation increased due to high bending i.e. low bending radius as illustrated in Fig. 2(d). This happened possibly due to the hot-cold barrier between the substrate and the surface of the droplet. This barrier is the origin of the rotation and with higher bending, the barrier increases. A detailed analysis can be found in our previous study [29]. The bending increased the sensitivity of the sensor. Apart from this, the effective contact angle (θ) also increases with decrease in bending radius. This also promotes the evaporation of droplet and thus temperature beyond 60°C is not preferred. The diameter of the sensing drop in this case was ~3 - 4 mm.

B. Sensing of Amylase

The experimental set-up described earlier is shown in Fig. 3(a). The amylase sensing experiments were performed by recording the resistance across the microdroplet sensor over time. The ~3 µl amylase was added into the starch-salt mixture droplet at t = 0s. The change in resistance at t =10s and t =30s was then calibrated with the concentration of amylase. The normalized variation in resistance R was considered as the sensor response, i.e. $\Delta R^N = (R - R_0)/R_0$, where R and R_0 denote the resistances at t = 30s and t = 10s respectively. The

optimal sensing temperature was identified after performing experiments for three different temperatures i.e. 26, 40, and 50°C, as illustrated in Fig. 3(b). It was observed that the sensor performance was best at 40°C when the amylase activity remains highest. The response of the sensor was higher for a higher concentration of amylase. The sensor was tested for 20-110 U/L of amylase concentration. The sensitivity of the sensor was calculated to be 0.88% (units/liter)$^{-1}$. The same sensor was also tested for biosamples i.e. amylase in human serum as shown in Fig. 3(c). The result from the bio-sample was found to be similar to that of the pathological results.

In this case, the selective hydrolysis of starch into maltose by α-amylase as discussed in previous works [30-32] ensures the specificity of the system. The change in the electrical resistance can be attributed to the starch-amylase reaction, which often leads to an excess of intermediate oxocarbenium ions [33-35]. The generation of excess ions is a primary reason behind the resistance change across the droplet. Apart from this, excess protonated carboxyl ions due to the catalytic ionizable groups of amylase also change the resistance across the microdroplet [33-35]. Hence, a higher change in *R* occurs due to the higher concentration of amylase. The active material of this sensor is a droplet and hence easy regeneration of the sensor is possible by replacing the droplet with a new one. The experiments are stable in ambient temperature and hence the effect of temperature can be neglected. Further, in the operating temperature, the evaporation of droplet is negligible. Moreover, the time required for the experiment is also very less for any significant evaporation effect to take place.

C. Readout Circuit

A flexible printed circuit board (PCB) was fabricated to accommodate the readout for the presented disposable sensor. Fig. 4 illustrates the optical image of the fabricated PCB on a flexible FR4 substrate. The presented sensor is resistive in nature and thus an appropriate excitation supply was required to measure the resistance changes due to the changes in the α-amylase concentration. For that purpose, a constant voltage excitation circuit with offset compensation and required amplification was designed (Fig. 5). The enhanced accuracy was ensured by the design complexity of the circuit having two OPA177 (Texas Instruments, Texas, USA) precision operational amplifiers, having very low offset voltage (25 µV maximum) and drift (0.3µV/°C). The precision amplifiers are part of the sensor conditioning circuit. In order to power the mentioned dual supply ±5 V op-amps, a voltage inverter was

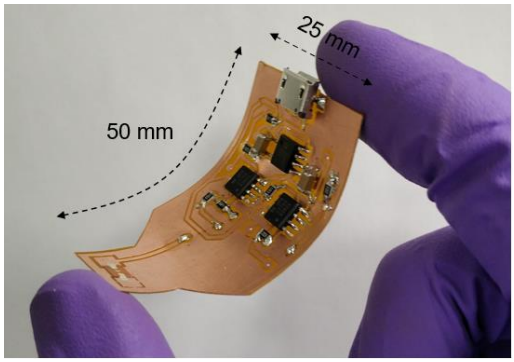

Fig. 4. Optical image of the fabricated flexible PCB.

designed using the ADM660 integrated circuit (Analog Devices, USA) to obtain the negative voltage of –5V from the

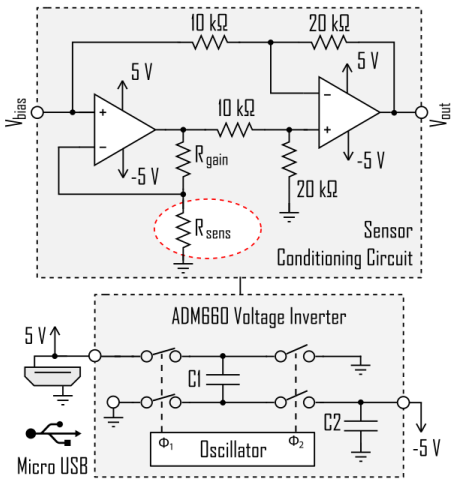

Fig. 5. Circuit schematic associated with readout of the sensor.

positive supply. The micro USB connector was used for the positive supply of +5 V, as shown in Fig. 5. The same micro USB connector was also used for the bias voltage using a resistor-based voltage divider. The simple relation between the output voltage to the resistive sensor value can be given by the following equation:

$$V_{out} = 2V_{bias}\left(\frac{R_{gain}}{R_{sens}}\right)$$

where V_{bias} = 0.5 V for appropriate biasing of the sensor and to avoid any noise due to electrolysis. The bias/applied voltage was kept low purposefully to avoid any electrochemical interference. The value of R_{gain} was kept 708 kΩ to maximize the output voltage range while keeping the values within the input common-mode voltage.

IV. Conclusion

The study shows that the rotational motion inside the droplet increases with the increase in temperature. Further, the bending of the substrate also increases the rotation inside the droplet. The droplet was made conductive by adding FeSO4 salt and then starch was added to make it specific towards amylase detection. It was observed that the sensor could detect amylase from 20 to 110 U/L concentration with a sensitivity of 0.88% (units/liter)$^{-1}$. Further, the best sensor performance was recorded at 40 – 45ºC (ΔT~18-23ºC). In addition, a flexible PCB was developed as sensor readout system to measure the concentration of amylase in blood serum. The sensor response was compared with biosamples and both the results were found to be in agreement with each other. The proposed sensor is a potential candidate for a POC device for the detection of amylase to diagnose pancreatic issues at an early stage. In the future, the heating element will be integrated in the flexible PCB to get the complete version of the POC device for wide use in healthcare applications.

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