A Portable Smartphone-based Platform with an Offline Image Processing Tool for Rapid Paper-based Colorimetric Detection of Glucose in Artificial Saliva

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Abstract

Paper-based sensors have great potential for use in a variety of areas, from environmental monitoring to clinical and point-of-care testings. Here, a microfluidic paper-based analytical device (µPAD) was integrated with a smartphone app capable of offline (without internet access) image processing and analysis for rapid colorimetric detection of glucose. A self-inking stamp was used to form hydrophobic channels on a piece of paper-towel due to its superior water absorption efficiency. As demonstrated, the developed sensor was employed for colorimetric detection of glucose in artificial saliva in the linear scope of 0-1 mM with a calculated detection limit of 29.65 µM. In addition, experimental results show that quantitative analysis of glucose with the proposed smartphone platform could be completed in less than one minute. The app developed for the smartphone platform is capable of extracting the color changing area with an embedded image processing tool which could address the problem of color uniformity in detection zones of µPAD. The total cost of a µPAD is less than $0.2. By integrating µPAD with a smartphone and user-friendly app together, the proposed smartphone-based platform could be used for the quantitative analysis of glucose with advantages such as portability, simple operation, rapid response, ultra-low cost, field-deployable, selectivity and sensitivity. The results show that the integrated platform has great potential to be used for non-invasive measurement of glucose in body fluids like a tear, sweat and saliva.

Index Terms

Lab-on-paper device, glucose detection, smartphone colorimeter, image processing, Android application, paper-based sensors.

I. INTRODUCTION

Paper-based sensors as novel analytical tools have a great potential to be used in a variety of fields ranging from environmental monitoring to clinical and point-of-care tests. These sensors have many advantages such as being disposable, practical, cost-effective, and user-friendly [1]. The first step in the prevention and treatment of a disease is the diagnosis. According to the World Health Organization (WHO), in order for diagnostic devices to be used in developing countries, they are expected to meet the following criteria, which are abbreviated as “ASSURED”; affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users [2], [3]. Paper-based sensors are thought to have the potential to meet these specified criteria. The concept of fabricating microfluidic paper-based analytical devices (µPAD) has attracted an increasing amount of attention after being introduced by Martinez et al. [4] and become popular in the detection of multiple analytes due to its potential applications in e.g. analytical and clinical chemistry.

Various detection methods such as chemiluminescence, fluorescence, electrochemical and colorimetric detection have been applied to µPADs for rapid, sensitive and selective analysis [5]. Among these detection methods, colorimetric detection has emerged as a useful tool for rapid qualitative analysis due to its ability to be used in remote locations or those with poor infrastructure where sophisticated tools or time-consuming steps cannot be afforded [6]. Moreover, the presence of the analyte in the detection zone causes a color change enabling visual readout which does not require special equipment for analysis and makes the system cheaper. The use of colorimetric detection in the paper-based analysis is promising as it enables portable, rapid and cost-efficient analysis with low sample and reagent consumption. However, this technology has some limitations in practice like poor color uniformity. The problem might stem from the case of that the solution flowing through the microchannels carries both the colorimetric reagents and unbound enzymes at the detection zone which degrades the color homogeneity. Various techniques have been introduced to improve color uniformity to a certain extent such as incorporation of chitosan on cellulose [7], paper oxidation [8] and fluid flow control [9].

Detection in most of the commercially available colorimetric diagnostic tools including pregnancy and urine test strips depends on visual inspection of discoloration in the detection zone. Visual inspection does not provide quantitative data due to poor
accuracy. The easiest way for quantification of a substance is to capture the visible color change using a camera and measure the color intensity using imaging software. Due to the latest advances in camera and sensor technologies, existing smartphones are nowadays fabricated with a low-power, high-performance processor and high resolution digital camera for image capturing even in dark conditions which lead them to be used in the quantitative analysis [10], [11]. Smartphone colorimeters are used in sample quantification based on color change with respect to concentration as in peroxide quantification [12] or with respect to time as in methylene blue degradation [13]. Recently, researchers have proposed several smartphone colorimeter designs, driven by applications such as water quality sensing [14], pH detection [15], glucose sensing [16], bisphenol-A detection [17] and food allergen testing [18]. In these designs, the smartphone colorimeters need to extract the color information depending on the color variation using color components of alternative color spaces like RGB (Red-Green-Blue), HSV (Hue-Saturation-Value), and L*a*b* (Lightness, Green-Red, Blue-Yellow) for quantitative color analysis [19]–[21]. In addition to color spaces, Joint Photographic Experts Group (JPEG) and RAW image formats were also studied to analyze the impact of the formats in the colorimetric analysis [13], [22]. In this study, a µPAD was used with a portable smartphone platform for colorimetric analysis of glucose.

A photopolymerizable polymer as printing material was used to print the µPAD on a paper towel with the help of a 3D-printed mold (Fig. 1A). Subsequently, HRP/GOx enzymes, chitosan and KI were placed in the detection zone for colorimetric detection of glucose. Glucose at various concentration was tested and the visible color change was captured using the portable printed mold (Fig. 1A). Subsequently, HRP/GOx enzymes, chitosan and KI were placed in the detection zone for colorimetric analysis [13], [22]. In this study, a µPAD was used with a portable smartphone platform for colorimetric analysis of glucose.

The major contributions of this paper can be summarized as follows:

1) A simple, cost-efficient, and sensitive smartphone-based platform integrated µPAD for rapid detection of glucose is reported.

2) A self-inking stamp along with a light-curable polymer hydrophobic in nature was used to form hydrophobic channels on a paper-towel.

3) The lateral flow speeds on paper-towel was compared to that of filter paper.

4) An Android app, named as GlucoSense, capable of offline (without internet access) image processing for extraction of the region of interest (ROI) was developed to make the platform more user-friendly for quantitative glucose analysis.

5) The smartphone-based platform integrated µPAD demonstrated high sensitivity (LOD=29.65 µM) and selectivity for glucose detection in artificial saliva. Therefore, it has great potential for non-invasive measurement of glucose in body fluids like tear, sweat and saliva.

The rest of this paper is organized as follows: the next section introduces the materials and methods for rapid colorimetric detection of glucose. Section III shows experimental results performed on µPAD with a portable smartphone platform and compares the performance with the current state-of-the-art studies. Finally, closing remarks are given in Section IV.

II. MATERIALS AND METHODS

A. Materials:

Phosphate buffered saline (PBS) (Sigma Aldrich, USA), glucose oxidase from Aspergillus niger (211 U/mg) (Sigma Aldrich, USA), peroxidase from horseradish (HRP, 325 U/mg) (Sigma Aldrich, USA), potassium chloride (KCl) (Sigma Aldrich, USA), potassium iodide (KI) (Sigma Aldrich, USA), chitosan - low molecular weight (Sigma Aldrich, USA), acetic acid - 2% (v/v) in H₂O (Sigma Aldrich, USA), D(+)-glucose (C₆H₁₂O₆) ≥99.5% (GC) (Sigma Aldrich, USA), paper-towel (Eagle photocell towel, Eagle Professional, Turkey), methacrylic/acrylic resin (HTM140 V2, EnvisionTEC, USA), artificial saliva (NeutraSal, Germany).

B. Formation of hydrophilic channels on paper:

Various stamps were designed using 3D CAD design software (SolidWorks version 2019, Dassault Systemes, USA) and printed with high precision using a 3D printer (M200, Zortrax, USA) with a 1.7 mm thick thermoplastic filament (Fig. 1B). The 3D-printed stamp was then mounted onto the base of a self-inking stamp (maximum impression area: 60 × 25 mm) using double-sided tape. The ink cartridge of the self-inking stamp was filled with a hydrophobic light curing methacrylic/acrylic resin and inserted into the self-inking stamp. The designed stamp was used to cast the resin on a paper-towel and filter paper (Whatman No. 1) to form the hydrophobic channels (Fig. 1A). Subsequently, the resin on both papers was light-cured for 20 s in a post-curing apparatus that delivers light in the 390-420 nm wavelength range (PCA 100, EnvisionTEC, USA). Next, the lateral flow behavior in hydrophobic channels formed on paper-towel and filter paper was assessed using a PBS solution colored with black ink. First, the printed device was cut proportionally using a pair of scissors and completely sealed with a transparent tape, except for the for sample insertion. 30 µl aliquots of colored solution were added to the sample insertion region of the device and the lateral flow was recorded using a smartphone camera.
Fig. 1. A schematic illustration of the fabrication process used to form hydrophobic channels on a piece of paper (A). 3D-printed molds (B) used with a self-inking stamp to form hydrophobic channels in different shapes (C). A light-curable polymer hydrophobic in nature was used as ink and diffused to both side of the paper ($C_{II}$, $C_{III}$, $C_{IV}$, $C_{V}$).

C. Fabrication of $\mu$PAD and glucose detection:

Detection zones of $\mu$PADs were modified for colorimetric detection of glucose. A detection mixture containing 180 U/ml GOx, 50 U/ml HRP, 3 mM KI and 1% (w/v) chitosan was prepared in PBS at pH 7. Then, 5 $\mu$l aliquots of the mixture was carefully and slowly injected into the detection zone of $\mu$PADs (Fig. 1A). The mixture was dried in the air for about 10 min at room temperature. Next, both sides of $\mu$PAD except for the region for sample insertion was sealed with a transparent tape. The colorimetric behavior of $\mu$PAD was assessed using PBS + glucose solutions. First, the contribution of GOx, HRP and chitosan to the discoloration in the detection zones was demonstrated by removing them one by one from the detection mixture. Basically, three mixtures each lacking one of the aforementioned components were prepared and added to the control regions of $\mu$PADs. The color change in the control regions of $\mu$PADs was assessed using 30 $\mu$l aliquots of PBS solutions containing 10 mM glucose. The color change in detection and control zones were imaged 1 min after the test solution was introduced into these regions. Then, 30 $\mu$l aliquots of test solutions containing glucose at varying concentrations (0.1, 0.25, 0.5, 0.75, 1, 5, 10, 15 and 25 mM) were introduced into the sample insertion regions of $\mu$PADs and allowed to reach both control and detection zones under lateral flow. Lastly, the sensor was used to detect glucose at varying concentrations (0.1, 0.25, 0.5, 0.75, and 1 mM) in NeutraSal’s artificial saliva using the design shown in Fig. 1C$_{IV}$.

D. Selectivity tests:

The selectivity of the paper-based sensor was evaluated in the presence of a number of interfering species such as sucrose (0.5 mM), urea (0.5 mM), lactate (0.5 mM), NaCl (10 mM), KCl (10 mM) and CaCl$_2$. The selectivity tests were conducted in artificial saliva.

E. Image processing:

A 3D printed apparatus was used with a smartphone to take the photos of $\mu$PAD for image processing and quantitative analysis. As stated above, first 30 $\mu$l aliquots of glucose + PBS solutions at varying concentrations (0.1, 0.25, 0.5, 0.75, 1, 5, 10, 15 and 25 mM) were introduced into the sample insertion regions of $\mu$PADs and then images of these $\mu$PADs were taken at 10, 20, 30, 40, 50 and 60 s time points using LG G4 (LG, South Korea) smartphone rear-facing camera with the specifications of 1/2.6 inch sensor size, 5312 $\times$ 2988 pixel resolution and 1.12 $\mu$m pixel size. To maintain consistency and repeatability, the smartphone camera was used in manual mode and settings for the ISO level (2200), shutter speed (1/10 s), focus level (f1.8) and white balance were kept constant during imaging. The LG G4 was purposely chosen because it is one of the few smartphones that support both JPEG and RAW file formats. RAW image contains linear RGB information about the scene radiance as it is minimally processed data from the digital camera sensor. The RAW images are converted to a commonly used format like JPEG for storage after applying post-processing methods, such as white balance, gamma correction, color
Fig. 2. Steps of colorimetric glucose detection with the GlucoSense app running on an Android smartphone.
space injection of 30 µl aliquots of colored solution into the insertion region of both devices, the lateral flow was video-recorded with multiple PADs made on the same sheet of paper. Next, the lateral flow speed of a colored PBS in µPADs made of both types of paper was assessed. Following injection of 30 µl aliquots of colored solution into the insertion region of both devices, the lateral flow was video-recorded with a camera. The observed lateral flow on paper-towel was drastically faster than it was on filter paper. The measured lateral flow speeds on paper-towel and filter paper were 6.3 and 1.2 mm/s, respectively. Basically, the paper is made of many small fibers with gaps between them. Aqueous solutions are pulled into these gaps through capillary action and the motion is further fueled with surface tension. Due to the fact that the porosity of paper-towels is optimized to suck water more efficiently, the speed of lateral flow on paper-towel was much higher than that on filter paper and this was one of the main reasons for choosing paper-towel as a substrate for making PADs. Glucose oxidase/peroxidase method was used for the detection of glucose. The detection mixture comprised GOx, HRP, chitosan and KI. Basically, GOx catalyzes the oxidation of β-D-glucose to D-glucono-1,5-lactone and also produces H₂O₂ as a by-product [24]. HRP uses the by-product H₂O₂ to catalyze the conversion of KI to iodine and thus produces a visual brown color. Chitosan has been shown to have peroxidase like activity and improve color uniformity and pixel intensity when used with the other components of the detection mixture [7]. The primary cause of this result may be that the chitosan provides a more suitable micro-environment for direct electron transfer between an enzyme and a reactive surface. The performance of the fabricated µPADs in the detection of glucose was determined using a portable smartphone-based platform that included a 3D printed cradle to remove the influence of the ambient light on the analysis. Poor color uniformity especially in lateral flow devices is one of the major problems of this technology. To address this problem, a custom-designed GlucoSense app capable of image processing was used to quantify the glucose concentration using a calibration curve extracted by processing the image dataset in MATLAB. Here, the design given in Fig. 1CII was used. Firstly, an image was cropped to decrease the image size as shown in Fig. 2d, e. The image was also converted to HSV and L*a*b* color channels which are more robust to illumination variation.

Image dataset, created by capturing nine concentration values at six-time points, was transferred to a computer to process in MATLAB (MathWorks, MA, USA) environment. The region of interest (ROI) in the detection zone which covers the color change caused by the reaction of mixtures was extracted by image processing algorithm including grayscale conversion, thresholding, binarizing, masking, contour detection and noise removal. The extracted ROI area was then masked with original images to calculate average R, G, B, H, S, V, L*, a*, b* values. These values were later used to plot the calibration curve with respect to concentration values.

F. Smartphone app: GlucoSense

In the previous subsection, a calibration curve was extracted by processing image dataset in computer. Here, we demonstrate a portable smartphone-based platform for rapid colorimetric detection of glucose controlled by an application, named as GlucoSense, developed in Android Studio. Contrary to other apps like [10], [12] which need an internet connection to transfer the images to process in the workstation, the image processing algorithm coded in MATLAB was re-coded in Eclipse for the Android platform with JAVA programming language, resulting in an app capable of image processing without internet connection (offline). Then, a simple and user-friendly interface was designed to provide rapid on-site quantitative monitoring when a fast analysis is required. Screenshots of the GlucoSense app given in Fig. 2 present the flow of running and quantification procedures. The app acquires the images by either accessing the gallery (internal storage) of the smartphone camera as shown in Fig. 2b or running camera to capture a new image. The user takes the ROI inside a square to crop a patch as given from Fig. 2c to Fig. 2e. Once the user taps the “calculate” button, the image processing algorithm is run on the patch and the ROI is extracted. Average R, V and L* values in the ROI are used to calculate the glucose concentration of the mixture and the result is displayed on the screen as shown in Fig. 2f.

III. RESULTS AND DISCUSSION

Various 3D printed stamps were used to cast methacrylic/acrylic resin on both a filter-paper (Fig. 1C1) and a paper-towel (Fig. 1C11) to form hydrophobic channels that control both the direction and the lateral flow of introduced solutions. The approach was facile and cost-effective and enabled printing of various shapes quite readily onto a sheet of paper. The resin used for printing was hydrophobic by nature and green in color. Impression with the self-inking stamp once or twice was enough to form hydrophobic barriers on both types of paper (Fig. 1CII,III,IV,V). Since there is no melting process to facilitate resin penetration into the paper, it is important to see the desired impression on both sides of the sheet prior to light-curing. All processes for a single µPAD can be completed in about 30 s and even less when multiple µPADs are made on the same sheet of paper. The lateral flow speed of a colored PBS in µPADs made of both types of paper was assessed. Following injection of 30 µl aliquots of colored solution into the insertion region of both devices, the lateral flow was video-recorded with a camera. The observed lateral flow on paper-towel was drastically faster than it was on filter paper. The measured lateral flow speeds on paper-towel and filter paper were 6.3 and 1.2 mm/s, respectively. Basically, the paper is made of many small fibers with gaps between them. Aqueous solutions are pulled into these gaps through capillary action and the motion is further fueled with surface tension. Due to the fact that the porosity of paper-towels is optimized to suck water more efficiently, the speed of lateral flow on paper-towel was much higher than that on filter paper and this was one of the main reasons for choosing paper-towel as a substrate for making µPADs. Glucose oxidase/peroxidase method was used for the detection of glucose. The detection mixture comprised GOx, HRP, chitosan and KI. Basically, GOx catalyzes the oxidation of β-D-glucose to D-glucono-1,5-lactone and also produces H₂O₂ as a by-product [24]. HRP uses the by-product H₂O₂ to catalyze the conversion of KI to iodine and thus produces a visual brown color. Chitosan has been shown to have peroxidase like activity and improve color uniformity and pixel intensity when used with the other components of the detection mixture [7]. The primary cause of this result may be that the chitosan provides a more suitable micro-environment for direct electron transfer between an enzyme and a reactive surface. The performance of the fabricated µPADs in the detection of glucose was determined using a portable smartphone-based platform that included a 3D printed cradle to remove the influence of the ambient light on the analysis. Poor color uniformity especially in lateral flow devices is one of the major problems of this technology. To address this problem, a custom-designed GlucoSense app capable of image processing was used to quantify the glucose concentration using a calibration curve extracted by processing the image dataset in MATLAB. Here, the design given in Fig. 1CII was used. Firstly, an image was cropped to decrease the image size as shown in Fig. 2d, e. The image was also converted to HSV and L*a*b* in order to analyze the impact of color spaces on the concentration-depended color change. In GlucoSense app, the
user needs to choose a square patch to maintain consistency in terms of coding (Figs. 2e, 3a) which needs to be processed with image processing algorithms to find the ROI. First, the square patch is converted to a grayscale image using the green channel of the image. It is then binarized with a threshold value calculated by the Otsu method [25]. The binarized image was given in Fig. 3b contained noises (white dots in the black area and black dots in the white area). These noises were removed in two steps by morphological operations (image processing) as shown in (Figs. 3c, 3d). The noise-free image in Fig. 3d is then masked with Fig. 3a to extract the ROI as given in Fig. 3e. These steps are also illustrated in Fig. 3f-j for 15 mM concentration which has more visible ROI. If the color change in the ROI is sharper, binarized image is less noisy as shown in Fig. 3g. After the ROI is extracted, average R, G, B values are calculated. The same ROI is applied to HSV and L*a*b* images resulting in nine color variables to analyze color change depending on the concentration variation.

The overall process given above is also employed to the RAW images. The calibration curves were obtained based on average

Fig. 3. Image processing steps for 0.1 mM concentration are given from (a) to (e) while these steps are shown from (f) to (j) for 15 mM.

Fig. 4. Glucose concentration-dependent intensity (normalized) calibration curves obtained with (red circles) and without (blue circles) image processing tool using JPEG (A1) images (n = 3). Time-dependent color intensity (normalized) change of different glucose concentration values (A2I1). The sensitivity (B3I) and selectivity (B1II) of the sensor were assessed in artificial saliva containing glucose from 0 to 1 mM and 20 mM interferents, respectively. A µPAD designed to measure glucose concentration in a saliva droplet (B3I2).
R, G, B, H, S, V, L\(^*\), a\(^*\), b\(^*\) values in the ROI as given in Fig. 4. Based on experimental investigations, the average of R, V and L\(^*\) gives more reliable characteristics as V and L\(^*\) are robust to illumination change. Calibration curves were obtained by processing images both in RAW and JPEG formats to analyze the impact of formats on quantitative evaluation. The relationship between the normalized intensity and the glucose concentration was linear in the range of 0 to 1 mM of glucose in both image formats and \(R^2\) of the calibration curve obtained using JPEG (\(R^2\): 0.9949) Fig. 4A\(_1\) was slightly better than that obtained using RAW images (\(R^2\): 0.9767). Therefore, JPEG image format can be used to adequately quantify the glucose concentration by the user as it is the most supported image format in smartphones and easy to process. Due to the higher water absorption efficiency of paper-towels, the color changing intensity remained almost constant for all test solutions only 30 s after glucose addition to the sample zone, which significantly shortens the assay time considering some recently published similar papers [26]–[30] Fig. 4A\(_{HI}\). It might be difficult for end users to choose the right region for more accurate analysis. To demonstrate the utility of the image processing tool, calibration curves of the same dataset were obtained using small circles that include the color changing areas. As can be seen in Fig. 4A\(_1\), the image processing tool improved the accuracy and thus the sensitivity of the measurement considering the change in the slopes of the calibration curves obtained between 0 and 1 mM of glucose (red circles: with image processing, blue circles: without image processing). In addition, each \(\mu\)PADs can be produced at least less than \(\$0.2\). To demonstrate the time-dependent color intensity change, images of the samples containing 0.5, 0.75, 1, 5, 10 and 15 mM glucose were taken at 10, 20, 30, 40, 50 and 60 s time points. As it can be easily seen in Fig. 4A\(_{HI}\), the relative color intensity of all samples became almost steady after 30 s, which suggests that the analysis can be completed in less than 1 min (Video S1, ESI). The analysis with filter-paper based \(\mu\)PADs is usually completed within 10 to 20 min [31]–[33]. In light of this result, glucose measurement time can be significantly shortened using the proposed strategy as compared to the duration of the analysis with \(\mu\)PADs made of filter papers. Various 3D printed stamps can be used to make different \(\mu\)PADs for different applications. Here, a \(\mu\)PAD was also designed for measuring glucose in a 10 \(\mu\)l of an artificial saliva droplet. This design could be more suitable for detection of glucose in saliva as the sample insertion zone can be placed on tongue for saliva suction (Fig. 4B\(_{II}\)). Due to its small size, 2 \(\mu\)l aliquots of the detection mixture was carefully and slowly injected into the detection zone of \(\mu\)PADs and allowed to dry at room temperature (\(~\)10 min). The artificial saliva droplets contained glucose at varying concentrations (0.1, 0.25, 0.5, 0.75, and 1 mM). Simply, the solution absorption region of the \(\mu\)PAD was touched to the droplet and the solution was sucked up through the channel and delivered to the detection zone through capillary force without the use of external equipment. A visible color was observed as soon as the sample solution reached the detection zone. The sensor gave a linear response for glucose concentration range between 0.1 and 1 mM (\(R^2\): 0.9819) in artificial saliva with a calculated LOD of 29.65 \(\mu\)M, which is low as compared to some papers found in literature, especially those using KI as indicator (Fig. 4B\(_{II}\)) [8], [26], [33]–[36]. The selectivity of the sensor was also tested against some interfering species and the results showed that the color intensity in the presence of these species was comparable to that of saliva with no glucose (Fig. 4B\(_{II}\)).

IV. Conclusion

In this paper, a \(\mu\)PAD was integrated with a portable smartphone-based platform for rapid, sensitive, selective and quantitative detection of glucose. The platform is user-friendly, ultra-low cost and field-deployable. A light curable polymer was successfully used to form hydrophobic channels on paper. A custom-designed 3D printed case with a smartphone was used for imaging to eliminate ambient light interference. Images were saved both in JPEG and RAW formats. All captured images were processed in MATLAB for precise extraction of the ROI to address one of the major limitations of this technology which is poor color uniformity and thus to improve the accuracy of the analysis. The obtained data were used to obtain the calibration curves. According to the results, both image formats can be used successfully for quantitative analysis due to negligible difference. Due to the higher water absorption efficiency of paper-towels, the color changing intensity remained almost constant for all test solutions only 30 s after glucose addition to the sample insertion zone, which enabled the completion of each test in less than 1 min. An Android app, named as GlucoSense, was developed by re-coding all image processing algorithm in JAVA to make the platform more user-friendly and allow the users to make offline quantitative glucose analysis. The app will be further improved to include various capabilities such as user-oriented self-calibration and running on ios platform. A different \(\mu\)PAD design was also used for glucose detection in artificial saliva to demonstrate that the proposed strategy can be adapted for various applications; the sensor demonstrated a linear response for glucose ranging between 0 and 1 mM with a calculated detection limit of 29.65 \(\mu\)M. Since the signal levels off at relevant blood glucose levels for people with diabetes, the integrated platform has more potential for non-invasive measurement of glucose in body fluids like tear, sweat and saliva, where the glucose concentration is much lower.

Acknowledgement

This research was supported by the scientific research projects coordination unit of Izmir Katip Celebi University (project no. 2019-ÖNAP-MÜMF-0004 and 2018-ÖDL-MÜMF-0021) and partly supported by the Scientific and Technical Research Council of Turkey (project no. 116E934 and 215E003).
CONFLICT OF INTEREST

There are no conflicts to declare.

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