Breath Acetone Based Detection of Diabetic Ketoacidosis

Kittaya Natanode[1] and Pimchanok Anuson[1]
Patipan Pakdeeto[1]*

[1] *Princess Chulabhorn Science High School Kamphaeng Phet 339 Moo 3, Kamphaeng Phet - Sukhothai Road, Nong Pling Sub-district, Mueang Kamphaeng Phet District, Kamphaeng Phet Province, 62000, Thailand*

**e-mail: pcshskp@sesapkpp.go.th*

Abstract

Diabetes mellitus has become a major global health challenge, affecting hundreds of millions of people worldwide and placing a significant burden on healthcare systems. Among its acute complications, diabetic ketoacidosis (DKA) is a severe and potentially life-threatening condition that requires rapid intervention. Conventional diagnostic approaches rely on invasive blood sampling, causing discomfort and limiting frequent monitoring. This study developed a non-invasive, portable colorimetric breath-testing prototype for early DKA risk screening through acetone detection in exhaled breath. The sensing platform was fabricated by immobilizing an optimized reagent system consisting of 0.10 M sodium nitroprusside (SNP), 1 M sodium hydroxide (NaOH), and 1 M glycine onto a glass fiber sheet substrate to enhance colorimetric response. The sensing strip was integrated into a 3D-printed device containing a silica gel moisture filter and an internal flow-stop channel to increase gas–sensor interaction time. When tested with simulated breath samples containing 3.3–132 ppm acetone, the alkaline-activated glass fiber strips exhibited distinct and stable yellow-to-purple color transitions. The prototype achieved an average visual reading accuracy of 88.14% in classifying acetone levels into safe, caution, and danger zones. Furthermore, the sensor showed good selectivity, with no observable color response to common respiratory interferents, including ammonia, methanol, and ethanol. This low-cost and painless screening approach demonstrates potential as an accessible tool for early DKA risk assessment and future development toward non-invasive monitoring systems.

keywords : Diabetic ketoacidosis (DKA), Breath acetone, Sodium nitroprusside (SNP), Colorimatric sensor, Non-invasive detection

Introduction

Diabetes mellitus has become a major global health challenge, affecting approximately 537 million adults worldwide according to the International Diabetes Federation (IDF). Among its acute complications, diabetic ketoacidosis (DKA) is a severe and potentially life-threatening condition caused by insulin deficiency, resulting in hyperglycemia, metabolic acidosis, and excessive ketone production. The increasing incidence of DKA, together with limited access to laboratory-based testing in developing regions, emphasizes the need for accessible and non-invasive screening methods. During DKA progression, fatty acid breakdown

produces ketone bodies, including acetoacetate, beta-hydroxybutyrate, and acetone. Acetone is a volatile compound released through exhaled breath, making it a potential biomarker for non-invasive ketone monitoring. Previous studies have shown a correlation between breath acetone levels and blood ketone concentrations. Colorimetric sensors based on sodium nitroprusside (SNP) have attracted attention due to their simplicity, affordability, and ability to provide visible color changes for acetone detection under alkaline conditions.

In this study, a portable colorimetric breath sensor was developed for early DKA risk screening. The sensing strip was fabricated by immobilizing 0.10 M sodium nitroprusside, 1 M sodium hydroxide, and 1 M glycine onto a high-porosity glass fiber substrate. The strip was integrated into a 3D-printed breathing device equipped with a moisture filter and flow-stop channel to improve sensing performance. The prototype was evaluated using simulated breath acetone concentrations ranging from 3.3 to 132 ppm and demonstrated clear color transitions with visual classification into safe, caution, and danger zones. This low-cost and non-invasive platform shows potential for accessible DKA risk monitoring, especially in areas with limited healthcare resources.

Methodology

1.) Preparation of the Colorimetric Sensing Strip.

Glass fiber sheets were cut into strips of the desired dimensions and used as the sensing substrate due to their three-dimensional fibrous structure, high porosity, excellent reagent retention, and chemical stability, which provide a more uniform colorimetric response than conventional cellulose-based paper. The sensing reagent was prepared by first dissolving 1 M glycine in distilled water, followed by the addition of 0.1 M sodium nitroprusside (SNP) under continuous stirring until a homogeneous solution was obtained. The reagent solution was uniformly applied onto the glass fiber strips using a micropipette and allowed to stand for 3 min to facilitate reagent absorption into the substrate, following the procedure adapted from Mahunnopnatee and Khunsrisuk (2025). Subsequently, 1 M sodium hydroxide (NaOH) was uniformly applied onto the coated strips using a micropipette to provide the alkaline condition required for the colorimetric reaction with acetone. Finally, the sensing strips were dried at room temperature in the dark until completely dry before being used in subsequent experiments.

2.) Preparation of Simulated Breath Acetone Samples.

Simulated breath acetone samples were prepared using analytical-grade acetone (99.5%). The target acetone concentrations (3.3, 6.6, 13.2, 33, 66, and 132 ppm) were selected based on previously reported breath acetone levels in healthy individuals, nutritional ketosis, and diabetic ketoacidosis (DKA), thereby covering clinically relevant concentrations for breath acetone analysis (Mager & Tabb, 1980; Anderson et al., 2021; Nasution et al., 2013).

The required acetone vapor volume for each concentration was determined using the dilution equation, $C_1V_1 = C_2V_2$, where C_1 is the acetone concentration in the vapor source, V_1 is the injected vapor volume, C_2 is the target concentration, and V_2 is the total volume of the sampling bag (5 L). A calculated volume of

acetone vapor was withdrawn from the headspace of a sealed 15 mL vial using a gas-tight syringe and injected into a 5 L zip-lock sampling bag. The bag was then gently mixed to ensure homogeneous vapor distribution before each experiment.

3.) Fabrication of the Portable Breath-Testing Device.

The portable breath-testing device was designed using Fusion 360 software and fabricated by three-dimensional (3D) printing. The device consisted of a sensing chamber for the glass fiber strip, a silica gel moisture filter to minimize humidity interference, and an internal flow-stop channel designed to prolong the interaction time between exhaled breath and the sensing strip.

4.) Colorimetric Detection Procedure

The prepared sensing strip was placed inside the portable device before each experiment. Simulated breath samples containing different acetone concentrations were introduced into the device under identical conditions. After exposure, the sensing strip was removed and photographed under controlled lighting conditions using the same camera position, background, and illumination. Each acetone concentration was tested in triplicate.

5.) Image Analysis

After each experiment, the sensing strip was photographed using a smartphone with the camera flash turned on under fixed lighting conditions to minimize variations in illumination. All images were captured within 3 min after exposure to acetone vapor because the colorimetric reaction may continue to develop under alkaline conditions, resulting in gradual changes in color intensity that could affect measurement accuracy. The images were then imported into ImageJ software and converted to 8-bit grayscale for quantitative image analysis.

The mean grayscale intensity of the sensing strip was measured before and after exposure to acetone vapor. A square region of interest (ROI) measuring 120 × 120 pixels was selected from the center of the reaction area, where the color distribution was visually uniform and free from edge effects. The same ROI size was applied to all samples. The mean grayscale intensity was measured at three independent locations within the reaction area, and the average of the three measurements was used for subsequent analysis to improve measurement reliability and reduce the influence of local color variation. The intensity change was calculated using the following equation:

$$Intensity\ change\ = \frac{I_0 - I}{I_0}$$

where I_0 represents the mean grayscale intensity of the sensing strip before exposure to acetone vapor, and I represents the mean grayscale intensity after exposure to acetone vapor. The calculated intensity changes were compared among different acetone concentrations to evaluate the colorimetric response of the sensing strip and classify acetone levels into Safe, Caution, and Danger categories.

Results and Discussion

Colorimetric Response of the Sensing Strip, the developed colorimetric sensing strip successfully responded to acetone vapor over the concentration range of 3.3–132 ppm. As shown in Figure 1, the sensing strip exhibited a distinct color transition from light yellow to purple with increasing acetone concentration.

This color change resulted from the reaction between acetone and sodium nitroprusside under strongly alkaline conditions in the presence of glycine, leading to the formation of a colored complex.

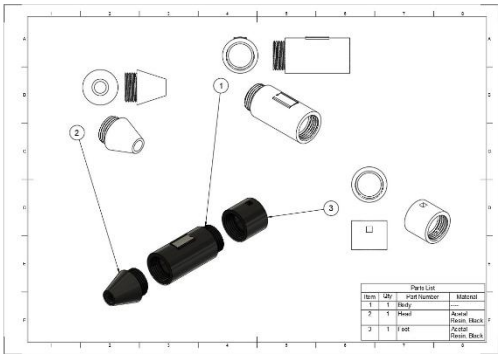
The gradual increase in color enabled clear visual differentiation among acetone concentrations corresponding to different DKA risk levels. Furthermore, the use of a glass fiber sheet as the sensing substrate provided excellent reagent retention and uniform reagent distribution, resulting in stable and reproducible color development throughout the experiment. Compared with conventional paper-based substrates, the glass fiber sheet demonstrated improved color homogeneity and enhanced sensing performance.

Table 1 Mean gray values of colorimetric sensing strips at various simulated breath acetone concentrations under alkaline and non-alkaline conditions.

Acetone Concentration (ppm)	Mean gray value (with base)	Mean gray value (without base)	Percentage change (%)
0	214 ± 0.01	223.944 ± 0.00	-
3.3	199 ± 0.05	165 ± 0.02	-
6.6	175 ± 0.08	141 ± 0.03	18.8
13.2	162 ± 0.03	127 ± 0.07	7.9
33	154 ± 0.09	107 ± 0.08	7.3
66	133 ± 0.07	102 ± 0.09	10.1
132	136 ± 0.08	98 ± 0.08	13.3
Pure acetone	120 ± 0.02	117.963 ± 0.07	6.9

The results in Table 1 demonstrate that alkaline treatment enhances the colorimetric response of the sensing strips. The mean gray value decreased with increasing acetone concentration, indicating a stronger color change under alkaline conditions compared with untreated strips. This confirms the importance of alkaline conditions in promoting the reaction between sodium nitroprusside and acetone.

Performance of the Portable Breath-Testing Device, a portable breath-testing prototype was successfully fabricated using Fusion 360 and three-dimensional (3D) printing technology. The developed device accommodated the colorimetric sensing strip together with a silica gel moisture filter and an internal flow-stop channel. The silica gel moisture filter effectively reduced humidity interference from exhaled breath before the sample reached the sensing chamber. This minimized moisture-related effects on the colorimetric reaction and improved color stability. Meanwhile, the flow-stop channel prolonged the contact time between acetone vapor and the sensing strip, allowing the chemical reaction to proceed more completely and resulting in a more distinguishable color change. These structural improvements enhanced the overall usability of the prototype while maintaining a compact, portable, and user-friendly design suitable for rapid point-of-care screening.



The developed prototype achieved an average visual classification accuracy of 88.14%, demonstrating that the colorimetric

response was sufficiently distinguishable for preliminary DKA risk screening. Although the proposed system is not intended to replace clinical diagnosis, the high visual accuracy indicates its potential as a practical non-invasive screening tool for identifying individuals who may require further medical evaluation.

To quantitatively evaluate the sensing performance, images of the sensing strips were analyzed using ImageJ after conversion into 8-bit grayscale images. The grayscale intensity before and after acetone exposure was measured, and the intensity change was calculated according to

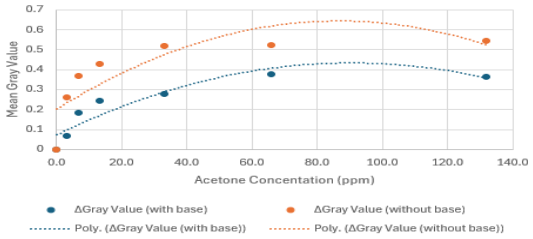
$$Intensity\ change = \frac{I_0 - I}{I_0}$$

where I_0 is the grayscale intensity before acetone exposure and I is the grayscale intensity after exposure. The grayscale analysis demonstrated that the intensity change increased with increasing acetone concentration, consistent with the visual observation of progressively darker purple coloration. This quantitative image-processing method reduced subjectivity associated with visual interpretation and provided a more reliable evaluation of the sensing performance. The combination of smartphone image acquisition under fixed flash illumination and grayscale analysis offers a simple, inexpensive, and reproducible approach for quantitative assessment without requiring specialized optical instruments.

Table 2 Comparison of Δ Gray Values of Acetone Sensing Strips with and Without Alkaline Treatment at Different Acetone Concentrations

Acetone Concentration (ppm)	Δ Gray Value (with base)	Δ Gray Value (without base)	Percentage change (%)
0	0 ± 0.00	0 ± 0.00	-
3.3	0.07 ± 0.04	0.26 ± 0.04	-
6.6	0.18 ± 0.05	0.36 ± 0.06	69.6
13.2	0.24 ± 0.03	0.43 ± 0.04	33.3
33	0.28 ± 0.05	0.52 ± 0.04	25.0
66	0.37 ± 0.04	0.52 ± 0.05	24.6
132	0.36 ± 0.02	0.54 ± 0.04	3.7
Pure acetone	0.34 ± 0.02	0.47 ± 0.02	7.7

As shown in Table 2, the Δ gray value increased with increasing acetone concentration for both sensing strips. The untreated strips consistently produced higher Δ gray values than the alkaline-treated strips, indicating a stronger colorimetric response under the same acetone concentration. However, the response of both strips approached saturation at higher acetone concentrations (66–132 ppm).



Note: The dotted lines represent polynomial trendlines of the mean gray values obtained from sensing strips with and without alkaline treatment at different acetone concentrations.

Selectivity Evaluation, the selectivity of the developed sensing strip was investigated using common volatile compounds found in exhaled breath, including ammonia, methanol, and ethanol. Under identical experimental conditions, these compounds produced no observable color change, whereas acetone generated a distinct purple coloration.

These results indicate that the optimized reagent system exhibits good selectivity toward acetone with minimal interference from common respiratory gases. This improves the sensor's reliability, supporting its potential for non-invasive DKA risk screening. Although the present study was performed using simulated breath samples, the obtained results demonstrate the feasibility of this approach for preliminary DKA risk screening. Future studies should evaluate the prototype using clinical breath samples from diabetic patients and investigate long-term sensor stability, quantitative calibration, and real-world performance to further improve its clinical applicability.

Conclusions

This study successfully developed a glass fiber-based colorimetric sensing strip for non-invasive breath acetone detection, serving as a potential screening platform for ketosis and diabetic ketoacidosis (DKA). Utilizing sodium nitroprusside under alkaline conditions, this portable, low-cost, and user-friendly tool exhibited a clear, concentration-dependent color change without requiring complex instruments. Furthermore, integrating smartphone-based image acquisition with grayscale intensity analysis enabled objective, semi-quantitative evaluation, minimizing visual subjectivity and demonstrating a practical fusion of chemical sensing and digital healthcare technology.

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