All Sciences Proceedings
http://as-proceeding.com/

5th International Conference on Applied Engineering and Natural Sciences

July 10-12, 2023 : Konya, Turkey

© 2023 Published by All Sciences Proceedings

https://www.icaens.com/

Development and Characterization of a Paper-Based Sensor for Colorimetric Urea Detection in Artificial Urine

Burak YAŞASIN[1] and Mustafa ŞEN[1*]

[1] *Department of Biomedical Engineering, Faculty of Engineering and Architecture, Izmir Katip Çelebi University, İzmir Turkey*

(mustafa.sen@ikcu.edu.tr) Email of the corresponding author

Abstract – Toxic nitrogen, which is formed as a result of the breakdown of proteins, is converted into non-toxic urea by the enzymes in the "urea cycle". While a small part of the produced urea is eliminated with sweat, a large part is removed from the body by the kidneys with urine. Therefore, urea determination in blood and urine provides important information about kidney functions. In this study, a colorimetric paper-based sensor was developed and characterized for use in urea detection. Basically, urea measurement was carried out by immobilization of urease enzyme on pH sensitive papers. The determination of urea was made by observing the changes in pH caused by ammonia with basic properties, which is formed as a result of the destruction of urea by urease. In the presence of different concentrations of urea, the colorimetric changes on the sensor were visualized with a camera and analyzed with the ImageJ program in the computer environment. In the first stage, a calibration curve showing the relationship between urea concentration and color intensity was obtained, and then the detection limit and sensitivity of the system were determined from this curve. Finally, the selectivity of the sensor was demonstrated by testing the different molecules involved.

Keywords – Paper-Based Sensor, Urea, Urine, Colorimetric Sensor

I. INTRODUCTION

Ammonia (NH_3) is formed as a result of deamidation and deamination reactions in mammals. NH_3 is a very strong water-soluble base and can raise the pH of the living environment to a harmful level. The ammonia formed, together with the CO_2 present in the blood as a product of metabolism, is converted by the liver into a non-toxic compound, urea. 90% of the urea formed is excreted by the kidneys in the urine, and the rest is excreted through the digestive system and skin. In a normal adult human, approximately 30 grams of urea is excreted in 24 hours. The urea excreted in the urine depends on the protein ratio, the urea synthesis rate in the liver, and the excretion rate of urea from the kidneys. Determination of urea has an important place for biomedical and clinical applications. An increase in urea concentration in the blood or a decrease in the urine is an important indicator of kidney dysfunction. (Hamlaoui et al. 2002). In this study, a paper-based biosensor capable of sensitive and selective urea detection was developed and characterized. The determination of urea was made by observing the changes in pH caused by basic ammonia formed as a result of the degradation of urea by urease. Litmus paper, which can respond to basic pH, was used in the construction of the sensor. Paper-based colorimetric sensors were produced by immobilizing the urease enzyme on this paper by physical adsorption technique. The sensors were tested with artificial urine samples containing urea at different concentrations, and the colorimetric changes that occurred as a result of the detection were visualized with a camera and analyzed with the ImageJ program in the computer environment. The results obtained revealed the potential of the developed sensor to be used in real samples.

II. Materials and Method

A. Design and Fabrication of µPADs

The Microsoft PowerPoint application was used to create the design for µPAD. At the bottom of the detection area, a thin hydrophilic channel was created, and the edges of the detection area were constructed with larger and longer hydrophobic barriers. The intended µPADs were printed using the wax printer on Whatman filter paper. After printing, µPADs were placed on a heater at +120°C for roughly 3–4 minutes to allow the solid ink to penetrate the chromatography paper's pores and thus form the hydrophobic barriers. To ensure a uniform heat transfer, the heater was covered with aluminium foil, and the paper was weighed down by a few kilograms. Next, 2.5 µL of urease solution was added to the detection area and allowed to dry for 10 minutes at room temperature. A litmus paper was placed on top of each µPAD and the two was sandwiched between two transparent tapes.

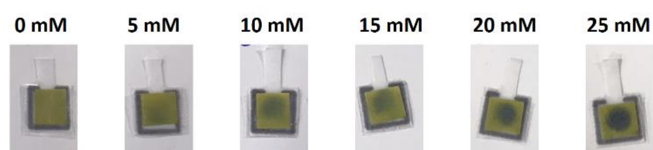

Fig. 1. Color change observed in the detection area of µPADs with artificial urine solutions containing urea at different concentrations.

B. Colorimetric Detection of Urea in Artificial Urine and Selectivity Test

Artificial urine solutions (120.9 mg NaCl, 16.5 mg $MgSO_4.7H_2O$, 9 mg $CaCl_2$ in 15 mL dH_2O, pH: 6.3) containing varying concentrations of urea (0, 5, 10, 15, 20 and 25 mM) were prepared. The sampling pad of µPADs were immersed into these urine solutions and the color change was recorded with a smartphone camera at different time points (0, 2, 4, 6, 8, 10 and 12 min). The images were then analyzed using ImageJ on a computer. A selectivity test was also carried out using various interferents (NaCl, glucose, sucrose, uric acid, dopamine, lactic acid, KCl, and urea) at 1 mM.

III. Results & Discussion

The fabrication procedure allowed us to make µPADs successfully. The design resulted in no liquid leakage and successful transfer of sample to detection areas for color change. Considering the amount of chemicals used for testing, the cost of analysis was considerably low. Once the µPADs were dipped into artificial urine sample with urea at different concentration, color change was observed in the detection areas of µPADs (Fig. 1). Basically, the enzyme urease catalyzes the conversion of urea to CO_2 and NH_3 which is a basic molecule that promptly changes the color of the litmus paper. The color change was imaged at different time intervals and analyzed using ImageJ software. The RGB intensity (weighted) of the images were used for analysis. As can be seen in Fig 2a, the highest RGB intensity was observed at 2 min. Next, the RGB intensity data of all samples with different concentrations of urea were used to obtain a calibration curve. As seen in Fig 2b, the relationship between the urea concentration and RGB intensity data was linear in the range of 5 to 25 mM with an R^2 value of 0.992. The limit of detection value of the sensor was calculated to be 2.39 mM (LOD = 3.3 σ/slope). In addition, the selectivity test proved the

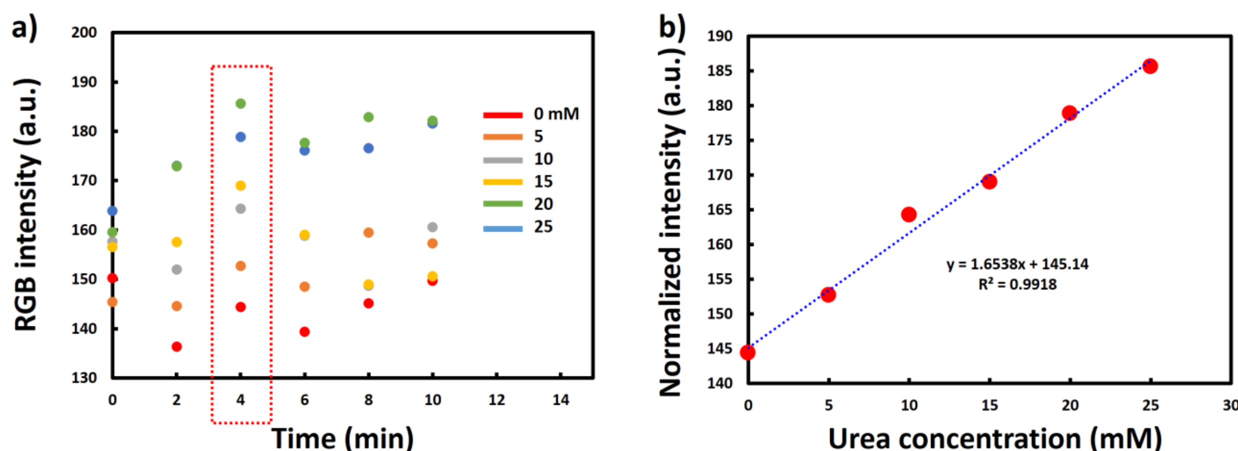

Fig. 2. Color change intensity at different time points (a) along with a calibration curve (b).

specificity of the μPADs for urea detection. Only when urea was present, the μPADs showed a noticeable color change, demonstrating their great potential for selective detection of urea.

IV. CONCLUSION

In conclusion, colorimetric detection of urea with μPADs in artificial urine showed promising results. The sensor had a low LOD and a linear response in a relevant concentration range. The selectivity test confirmed the urea detection specificity of the μPADs. The sensor thus has a great potential to be applied to real samples for diagnostic purposes.

ACKNOWLEDGMENT

The study was supported by supported by the Scientific and Technical Research Council of Turkey (TUBITAK 2209-a).

REFERENCES

[1] Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A., & Vernekar, S. N. "Markers of renal function tests." *North American journal of medical* sciences, vol. 2(4), pp. 170, 2010.

[2] Doğan V, Yüzer E, Kılıç V, Şen M. "Non-enzymatic colorimetric detection of hydrogen peroxide using a μPAD coupled with a machine learning-based smartphone app." *Analyst*, vol. 146, pp. 7336-7344, 2021.

[3] Mercan ÖB, Kılıç V, Şen M. "Machine learning-based colorimetric determination of glucose in artificial saliva with different reagents using a smartphone coupled μPAD." *Sensors and Actuators B: Chemical.,* vol. 329, pp. 129037, Feb 2021.

[4] Yüzer E, Doğan V, Kılıç V, Şen M. "Smartphone embedded deep learning approach for highly accurate and automated colorimetric lactate analysis in sweat." *Sensors and Actuators B: Chemical*., vol. 371, pp. 132489, Nov 2022.

[5] Gölcez T, Kilic V, Şen M. "A portable smartphone-based platform with an offline image-processing tool for the rapid paper-based colorimetric detection of glucose in artificial saliva." *Analytical Sciences*, vol. 37, pp. 561-567, Apr 2021.

[6] Şen M, Yüzer E, Doğan V, Avcı İ, Ensarioğlu K, Aykaç A, Kaya N, Can M, Kılıç V. "Colorimetric detection of H_2O_2 with Fe_3O_4@ Chi nanozyme modified μPADs using artificial intelligence." *Microchimica Acta.,* vol. 189, pp. 373, Oct 2022.