Microfluidics Paper-based Analytical Device for Fluorescence Determination of Hydrogen peroxide based on Thiamine-Hematin Reaction

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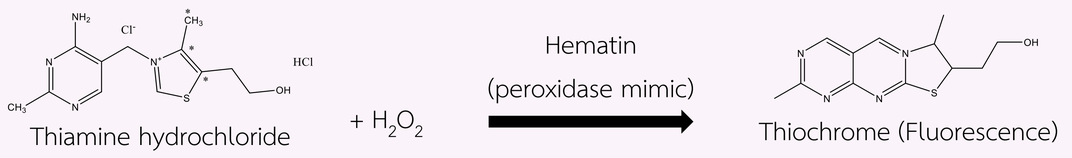
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ABSTRACT

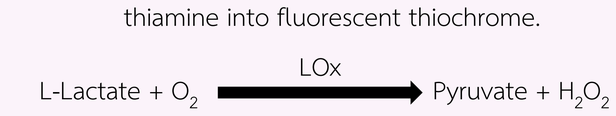
Hydrogen peroxide (H_2O_2) determination is an essential in clinical and biochemical applications, particularly in diagnostics. H_2O_2 serves as a crucial signaling molecule, its overproduction leads to oxidative stress, causing significant cellular damage to DNA, proteins, and lipids. This research aims to develop a rapid and cost-effective microfluidic paper-based analytical device (μPAD) for H_2O_2 detection based on fluorometric reaction of thiamine (Vitamin B1) and hematin. In this system, hematin is utilized as a peroxidase mimic to decompose H_2O_2, which in turn oxidizes thiamine into fluorescent thiochrome. The developed device exhibited a linear range of 1 - 30 μM with a regression coefficient (R^2) of 0.9961. A limit of detection (LOD), calculated based on $3SD_{blank}$, was 2.5 μM. Furthermore, the μPAD was applied for the detection of lactate via lactate oxidase enzyme. Based on the incubation with enzyme, the linear range of 10 - 100 μM lactate with a coefficient of determination (R^2) of 0.9980 can be obtained with the LOD of 10.0 μM (defined as the lowest detectable concentration within the linear range). The developed μPAD shows great potential for future application in on-site analysis and point-of-care testing.

INTRODUCTION

Hydrogen peroxide is a critical biomarker for oxidative stress and various clinical conditions. In this study, a portable μPAD was developed utilizing hematin as a cost-effective peroxidase mimic to catalyze the reaction between thiamine and H_2O_2, producing fluorescent thiochrome in **Scheme 1**. This sensing platform can be extended to detect other analytes, such as L-Lactate via enzymatic reaction in **Scheme 2.**



Scheme 1. Reaction for H_2O_2 detection based on the hematin-catalyzed oxidation of thiamine into fluorescent thiochrome.



Scheme 2. Enzymatic oxidation of lactate by lactate oxidase (LOx) to produce H_2O_2.

EXPERIMENT

➢ Analytical Procedure using Fluorescence Spectroscopy

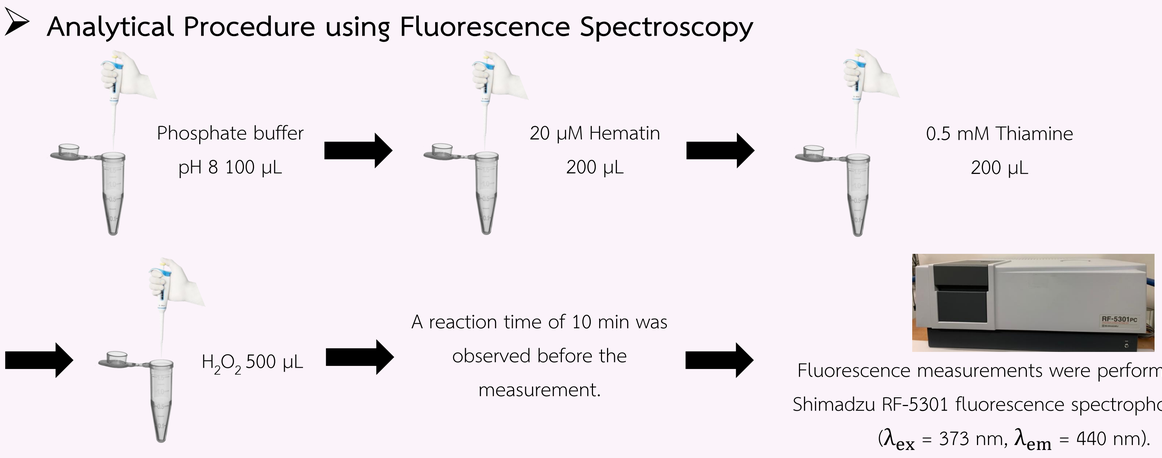


Fig 1. Analytical procedure for fluorescence measurement using a Shimadzu RF-5301 spectrophotometer.

➢ Fabrication of paper-based analytical device (μPAD)

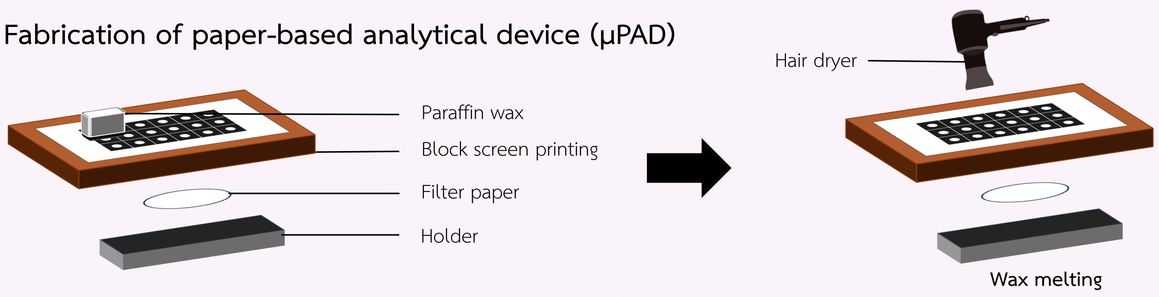


Fig 2. Fabrication of paper-based analytical devices (μPADs) using block screen printing.

➢ Analytical Procedure using the developed μPADs sensor

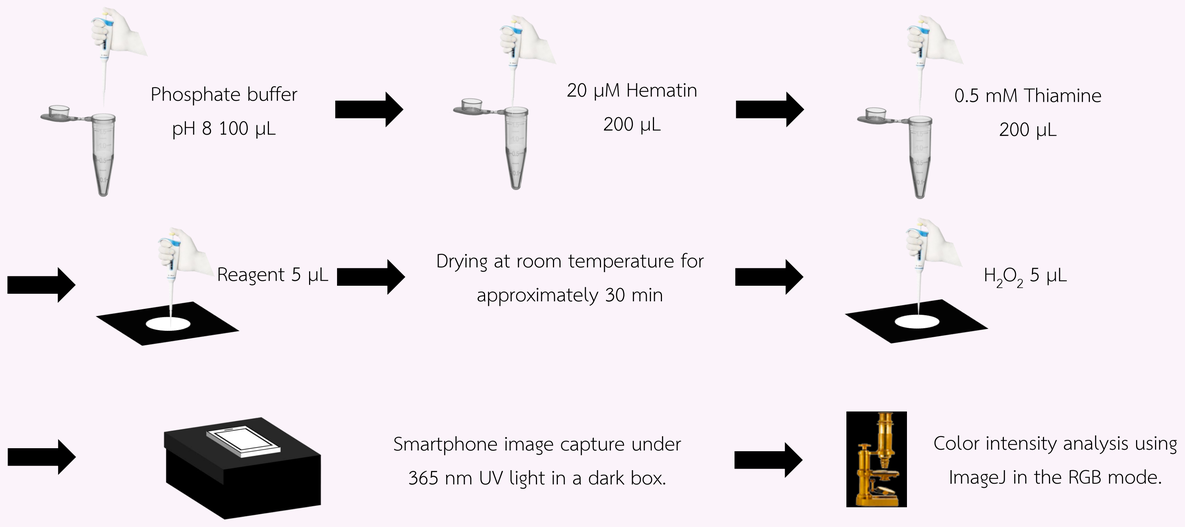


Fig 3. Step-by-step operation of the developed paper-based device.

RESULTS

➢ Determination of hydrogen peroxide (H_2O_2)

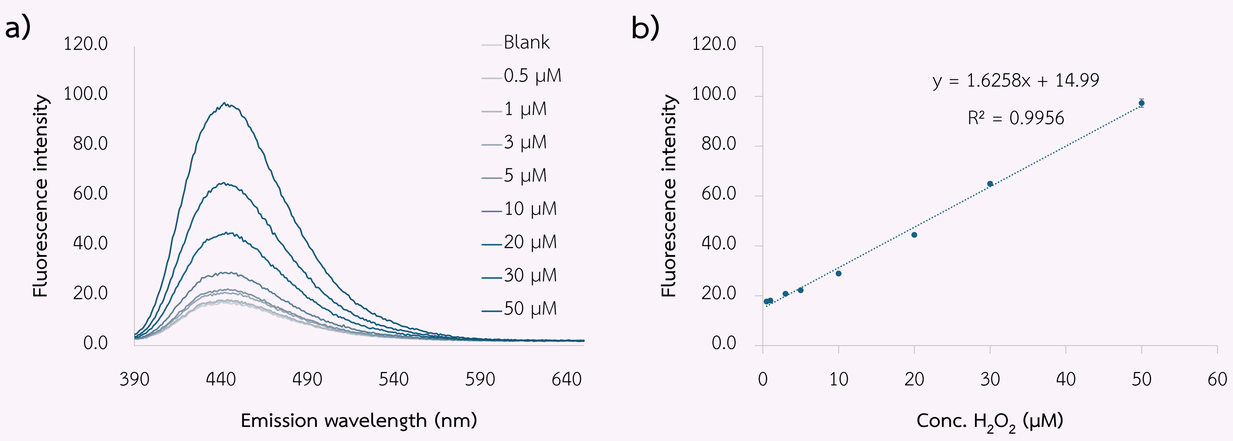


Fig 4. Detection of hydrogen peroxide using standard fluorometry **a)** Fluorescence emission spectra (λ_{ex} = 373 nm), **b)** Calibration curve for H_2O_2 detection based on the fluorescence intensity at 440 nm.

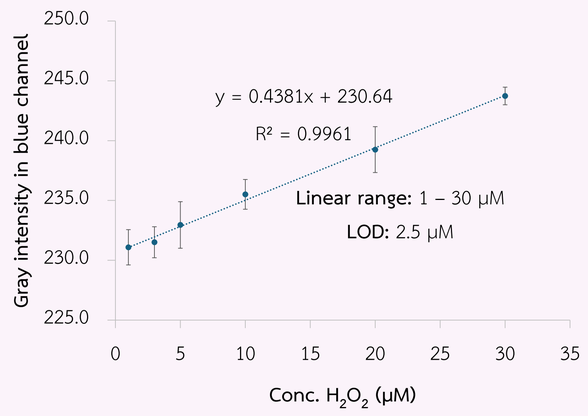


Fig 5. Calibration curve for H_2O_2 detection on the developed μPADs. The color intensity was quantified from smartphone images using ImageJ software in the blue channel of the RGB color.

➢ Determination of lactate

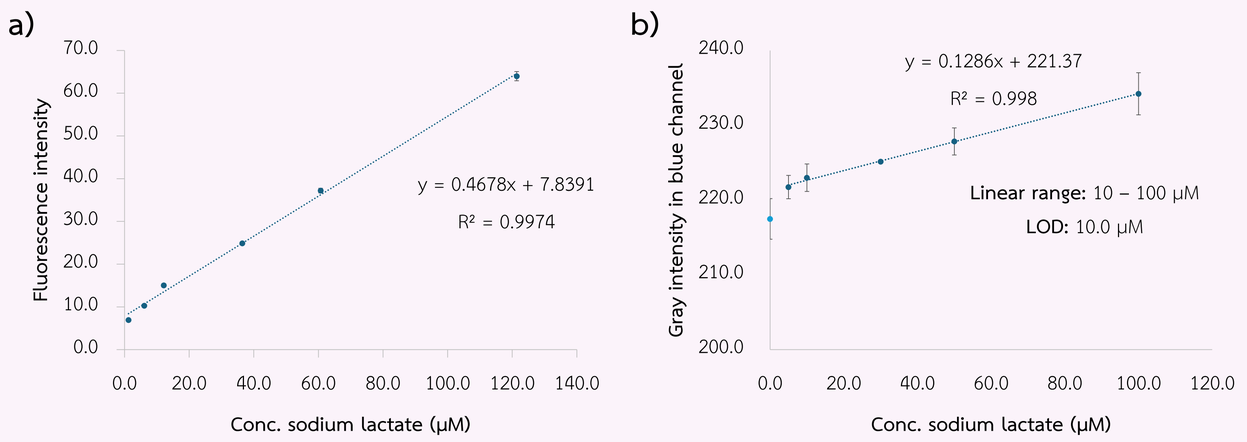


Fig 6. Calibration graph for lactate detection. **a)** Standard fluorescence intensity at 440 nm. **b)** Blue color intensity from smartphone images on μPAD.

CONCLUSIONS

- Development of a rapid and low-cost method for H_2O_2 detection using μPADs based on the reaction of thiamine-hematin-H_2O_2.
- A linear range of 1 – 30 μM with a limit of detection (LOD) of 2.5 μM.
- The μPAD was successfully applied for lactate determination via enzymatic oxidation by lactate oxidase, with a linear range of 10 – 100 μM, and LOD of 10.0 μM.
- Future work will focus on evaluating the sensor's accuracy in complex samples such as milk for on-site sensing applications.

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REFERENCES

[1] Li, J., & Dasgupta, P. K. (2000). Measurement of Atmospheric Hydrogen Peroxide and Hydroxymethyl Hydroperoxide with a Diffusion Scrubber and Light Emitting Diode-Liquid Core Waveguide-Based Fluorometry. Analytical Chemistry, 72(21), 5338–5347.
[2] Zhu, Q. Z., Li, Q. G., Lu, J. Z., & Xu, J. G. (1996). Application of Thiamine as A Fluorogenic Substrate in the Determination of Hydrogen Peroxide Based on the Catalytic Effect of Hemin. Analytical Letters, 29(10), 1729–1740.