Detection of Hydrogen peroxide using Fluorescence technique

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ABSTRACT

This study focuses on the measurement of hydrogen peroxide using a fluorescent technique. In this experiment, 4-hydroxyphenylacetic acid was used as the substrate, and horseradish peroxidase enzyme was used as the catalyst. The reaction time was set to 10 minutes in a phosphate buffer medium with a pH of 7.4 and a concentration of 10 millimolar. The fluorescent intensity emitted was measured in the wavelength range of 295 to 550 nanometers, with the peak emission detected at 414 nanometers. A standard curve was then constructed by plotting the fluorescent intensity against the concentration from 0 to 100 micromolar. It was found that the linear range was between 10 and 60 micromolar, with an R^2 value of 0.9918. Following this, hydrogen peroxide levels in real samples will be measured.

INTRODUCTION

Hydrogen peroxide (H_2O_2) is an essential compound in both biological and industrial processes, serving as an oxidizing agent with applications in disinfection, bleaching, and as a signaling molecule in various metabolic pathways. However, due to its potential toxicity at high concentrations, accurate and sensitive detection of hydrogen peroxide is crucial. Traditional detection methods, such as colorimetric and electrochemical techniques, often require complex instrumentation or extended processing times.

A more innovative approach combines enzymatic reactions with fluorescence detection. In this method, hydrogen peroxide reacts with horseradish peroxidase (HRP) in the presence of 4-hydroxyphenylacetic acid (4-HPAA), generating a measurable fluorescence signal. The enzyme HRP catalyzes the oxidation of 4-HPAA by hydrogen peroxide, producing a fluorescent product that can be detected at specific wavelengths. This technique offers high sensitivity, selectivity, and real-time detection, making it particularly effective for monitoring hydrogen peroxide concentrations in diverse environments, from biological systems to environmental samples.

This poster introduces a method for measuring hydrogen peroxide using enzymatic reactions coupled with fluorescence detection. Future work will focus on applying this method to real sample.

OBJECTIVES

- To study enzymatic reactions (Hydrogen peroxide-Horseradish peroxidase-4-Hydroxyphenylacetic acid reaction).
- To detect hydrogen peroxide using fluorescence technique.
- To implement enzymatic reactions combined with fluorescence detection for hydrogen peroxide detection in real samples.

METHODOLOGY

Enzyme + H_2O_2 + Substrate → **Fluorescent compound**

Horseradish peroxidase (HRP) — precursor

4-Hydroxyphenylacetic acid (4-HPAA)

reduction-oxidation reaction

dimer 2,2-dihydroxybiphenyl-5,5diacetate

Step 1 Preparation of Phosphate buffer pH 7.4

Step 2 Preparation of various concentration of Hydrogen peroxide

Step 3 Preparation of Working solution

Step 4 The experiment of the H_2O_2-HRP-4HPAA reaction using a spectrofluorometer.

1 5 10 20 40 60 80 100 (μM)

Hydrogen peroxide in 10.00 mL

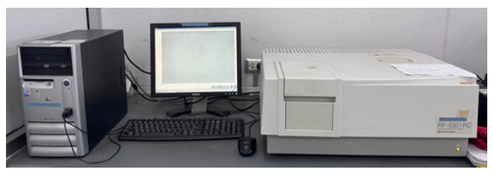
HRP 10 mM, 2.500 mL + 4-HPAA 10 mg/mL (234 units/mg), 25.00 μL

Reagent in 25.00 mL

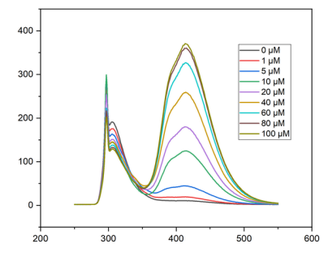
500 μM

500 μM

Reaction time for 10 minutes

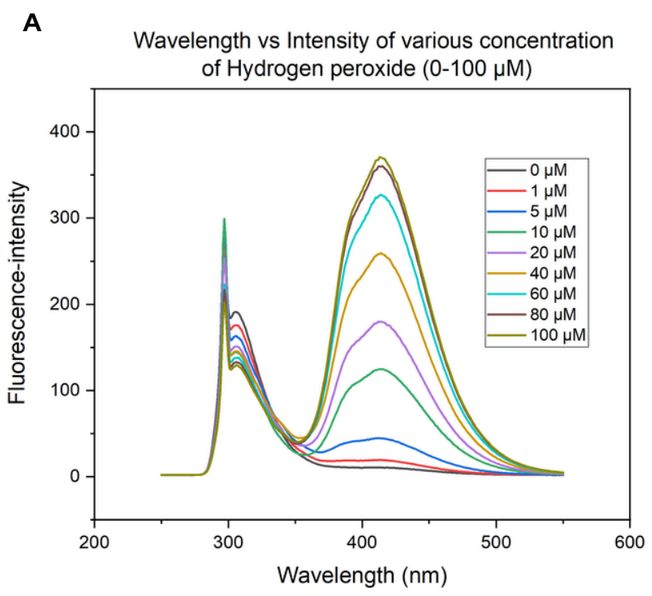


Spectrofluorometer (RF-5130PC)



Fluorecence spectrum

RESULTS AND DISCUSSION



(A) Fluorescence spectrum
(B) Calibration curve of H_2O_2 concentration (0-100 μM) and fluorescence-intensity unit at wavelength 414 nm.

(A) From the spectrum data of fluorescence intensity and wavelength obtained from measurements, the maximum wavelength observed is 414 nm. A calibration curve was then plotted between the concentration of H_2O_2 and the fluorescence intensity at the maximum wavelength of 414 nm. It was found that the measurement could be performed in the concentration range of 1–100 μM, with the most linear range observed between 10–60 μM.

(B) The linear correlation showed an R-square value of 0.9918. The limit of detection (LOD) was determined to be 0.7527 μM, and the limit of quantitation (LOQ) was 2.509 μM. These LOD and LOQ values indicate that the system or experimental technique has a good capability for detecting and quantifying the target substance.

CONCLUSIONS

- The fluorescent intensity emitted was measured in the wavelength range of 295 to 550 nanometers, with the peak emission detected at 414 nanometers.
- A calibration curve was constructed by plotting the fluorescence intensity against concentration from 0 to 100 μM. The linear range was found to be between 10 and 60 μM, with R^2= 0.9918, LOD= 0.2527 μM, and LOQ= 2.509 μM.

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