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Electrochemical Detection of Horseradish Peroxidase at Zeptomole Level

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Abstract: An electrochemical method for determination of horseradish peroxidase (HRP) was developed using a capillary catalytic system. HRP can be measured in several minutes with a detection limit of 4.8×10^{-12} mol/L or 47 zmol (*S/N*=3).

Keywords: Electrochemical detection, catalytic reactor, horseradish peroxidase.

Horseradish peroxidase (HRP) is the archetypal heme peroxidase. The determination of HRP is considerably important in clinical chemistry and analytical biochemistry, because HRP is the commonly used enzyme label for immunological detection systems[1]. We developed a novel method based on its catalytic reaction. A capillary catalytic reaction system was designed (**Figure 1**). In the assay, both HRP and H_2O_2 are injected into the polyacrylamide-coated injection capillary (10) by electromigration. After HRP is introduced into the reaction capillary (11), it catalyzes the reaction of enzyme substrate, *i.e*. the reaction of 3,3′,5,5′-tetramethyl-benzidine (TMB(Red)) from a reservoir (4) by raising liquid level and H_2O_2. The products are TMB (Ox) and H_2O. TMB (Ox) can be determined with amperometric detection on a carbon fiber microdisk bundle electrode at the outlet of the reaction capillary. Since the concentration of TMB(Ox) is much higher than that of HRP due to the enzyme amplification, the limit of detection (LOD) should be very low. The optimum conditions obtained are 2.0×10^{-3} mol/L H_2O_2 + 1.5×10^{-2} mol/L borate (pH 7.4) for the run buffer, 2.0×10^{-4} mol/L TMB(Red) + 2.0×10^{-2} mol/L citrate phosphate (pH 5.0) for the substrate solution, 40 cm liquid height for the liquid pressure, 20 kV for the run voltage and 0.1 V (*vs* SCE) for the detection potential. In this method, the response for a series of ten injections of 1.2×10^{-9} mol/L HRP resulted in a relative standard deviation of 2.2% for the peak area. The linear range is from 1.2×10^{-10} to 2.4×10^{-8} mol/L. The detection limit of concentration and mass are 4.8×10^{-12} mol/L and 47 zmol, respectively. Using this method, the activity of a commercial HRP product was measured to be 263 U/mg within ten minutes, which agrees with the value (250 U/mg) given by the specification. The recovery is between 95% and 102%.

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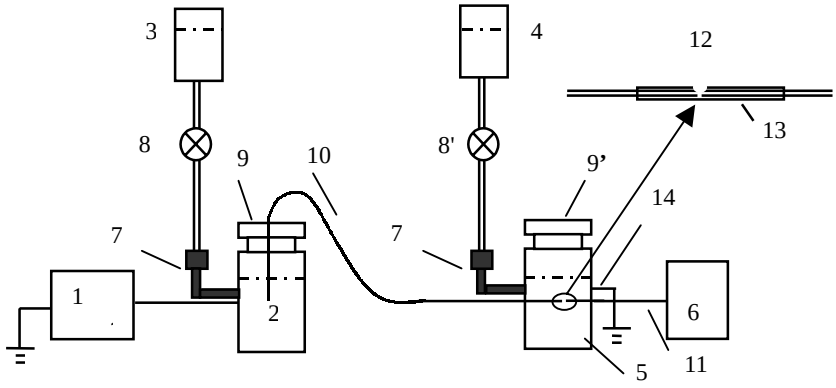
Figure 1 Overview of the capillary catalytic reaction system



1. high-voltage power; 2. run buffer reservoir; 3. liquid pressure buffer reservoir; 4. liquid pressure substrate reservoir; 5. catalysis reactor; 6. electrochemical detector; 7 metal tubing; 8 and 8′ switch; 9 and 9′. rubber cover; 10. run capillary; 11. reaction capillary; 12. hole; 13. syringe needle; 14. Pt wire.

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Reference

1. K. H. Milby, *Enzyme-Mediated Immunoassay* (T. T. Ngo and H. M. Lenhoff, eds.), Plenum Press, New York / London, **1985**, p.325.

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