[PD4-12] [10/19/2000 (Thr) 15:00 - 16:00 / [Hall B]]

Amperometric Enzymatic Determination of Ethanol Using Potassium ferrocyanide as the Mediator

Kim EK°, Hahn Y

Department of Chemistry, Sangmyung University, Seoul 110-743, Korea

Alcohol oxidase specifically reacts with alcohols producing aldehydes and hydrogen peroxide. Hydrogen peroxide itself can be measured amperometrically at a relatively high positive potential but ascorbic acid and other drugs in the biological fluids may interfere. In this study potassium ferrocyanide(Fo) is coupled to hydrogen peroxide using peroxidase(PO) enzyme so that the rate of overall enzymatic reactions is determined by the alcohol oxidase(AO) reaction. Then potassium ferricyanide(Fi) produced by the peroxidase reaction is measured at - 50 mV which is low enough not to have interference. A thin layer glassy carbon flow cell is connected to the flow injection system for the measurement. According to the kinetic studies, the enzymatic reactions reached to equilibrium in two minutes at 25oC with a solution containing 9.0 U/ml AO, 50 U/ml PO, 2.5 x 10-4 M Fo and 2.5 x 10-5 M ethanol. Ethanol was also completely converted to Fi. The present analytical method offers the advantage of quantification using Fi standard addition and requiring small volume(200 μl) of solution per measurement.

[PD4-13] [10/19/2000 (Thr) 15:00 - 16:00 / [Hall B]]

Spectrophotometric Studies of Hydrogen peroxide -Producing Oxidase Reactions Using Tetramethylbenzidine as the Mediator

Lee HY°, Hahn Y

Department of Chemistry, Sangmyung University, Seoul 110-743, Korea

Glucose, cholesterol and ethanol are the clinically important substances which are also the substrates of glucose oxidase(GO), cholesterol oxidase and alcohol oxidase, respectively. These oxidases commonly produce hydrogen peroxide(HP) which can be coupled to 3,3',5,5'-tetramethylbenzidine(TMB) speedily by peroxidase(PO). As the preliminary studies to develop a visual sensor for ethanol, we have studied absorption spectra of the reaction mixtures(reacting period: 2 min) consisting of 0.4~1600μM HP, 0.4 mM TMB and 36 U/ml PO. At the low concentrations of HP(0.4~40μM), the reaxtion mixtures developed yellow color and showed λmax at 405nm. At the higher concentrations the solutions turned into green ~ blue green showing two λmax at 370nm and 650 nm. Similar spectrophotometric studies were performed with the mixtures of 0.16~112μM glucose, 0.4U/ml GO, 0.4 mM TMB and 36 U/ml PO reacted for one minute. At the glucose concentrations less than 8μM, yellow colors were developed showing λmax at 400nm At the higher concentrations, two λmax at 370nm and 650 nm were also appeared. Absorbances at λmax were linearly increased as the concentrations of HP or glucose were increased. Spectrophotometric studies of ethanol-alcohol oxidase reaction will be presented.

[PD4-14] [10/19/2000 (Thr) 15:00 - 16:00 / [Hall B]]

Differential Pulse Polarographic Studies of Cephalosporins

Ra EY°, Hahn Y