

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

### **Amperometric Enzymatic Determination of Ethanol Using Potassium ferrocyanide as the Mediator**

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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 25°C with a solution containing 9.0 U/ml AO, 50 U/ml PO,  $2.5 \times 10^{-4}$  M Fo and  $2.5 \times 10^{-5}$  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  $\mu$ l) of solution per measurement.

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

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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 ~ 1600  $\mu$ M HP, 0.4 mM TMB and 36 U/ml PO. At the low concentrations of HP(0.4 ~ 40  $\mu$ M), the reaction mixtures developed yellow color and showed  $\lambda_{max}$  at 405nm. At the higher concentrations the solutions turned into green ~ blue green showing two  $\lambda_{max}$  at 370nm and 650 nm. Similar spectrophotometric studies were performed with the mixtures of 0.16 ~ 112  $\mu$ M glucose, 0.4U/ml GO, 0.4 mM TMB and 36 U/ml PO reacted for one minute. At the glucose concentrations less than 8  $\mu$ M, yellow colors were developed showing  $\lambda_{max}$  at 400nm. At the higher concentrations, two  $\lambda_{max}$  at 370nm and 650 nm were also appeared. Absorbances at  $\lambda_{max}$  were linearly increased as the concentrations of HP or glucose were increased. Spectrophotometric studies of ethanol-alcohol oxidase reaction will be presented.

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### **Differential Pulse Polarographic Studies of Cephalosporins**

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