CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Detection of Hydrogen Peroxide in Biological Samples

Purpose
Detection of hydrogen peroxide in complex biological samples, such as serum, plasma, and urine. This is only a preliminary report and has not been verified by BAS R&D.

Problem
H_2O_2 is detected at a platinum electrode, maintained at +0.5 V relative to an Ag/AgCl reference electrode. H_2O_2 dissolved in water can be quantitated with ease and precision (Capsule 128). However, quantitation of H_2O_2 in a biological matrix poses a problem. The various proteins as well as the smaller components can adsorb to the electrode surface, thereby passivating the electrode surface, or mask the H_2O_2 oxidation signal.

Reference
Surface-Modified electrochemical Detector for Liquid Chromatography, G. Sittampalam and G.S. Wilson, Anal. Chem., 1983, 55: 1608-1610.

This report utilizes a cellulose acetate (CA) film to select against the detection of various analytes while still maintaining the ability to detect H_2O_2. The CA film also prevents electrode passivation by protein adsorption.

Electrode Modification
The platinum electrode, TL-10, was cleaned, conditioned, and a CA film formed before the cell was assembled.

System
A flow injection system was used and consisted of a SP-8700 Solvent Delivery System, pulse damper column, injector and an LC-4B Electrochemical Detector. BAS 200 and BAS 400 systems have successfully been used in the flow injection mode. Details can be supplied if required.

Conditions
Mobile Phase: 0.1 phosphate, pH 7.4, containing 2 mM EDTA. Flow rate was 0.5 ml/min.
Potential: 0.8 V vs. Ag/AgCl.

Results
The CA film attenuated the signal from the oxidation of H_2O_2 by approximately 20-fold, as compared to a bare electrode. However, the CA film protected the electrode from passivation by proteins and selected against small molecules like ascorbic acid.

Table 1. Effect of BSA on the sensitivity of a Pt Electrode to H_2O_2

	Slope of Calibration Curve nA/n mole	
Solution	bare Pt	CA-coated Pt
H_2O_2	100.7	58.6
H_2O_2 + 0.2% BSA	49.5	55.8

Table 2. Response of a CA-coated Pt Electrode to H_2O_2

Solution	Peak Current (nA)
H_2O_2	54.5
H_2O_2 + glucose	55.6
Ascorbate	2.0
Glucose	2.2

The reponse of the CA-coated electrode to H_2O_2 was monitored before and after injection of undiluted serum. The electrode response was unchanged by the exposure to the serum, indicating that the electrode was not passivated by the serum proteins.

Summary

Although this preliminary report indicates that the H_2O_2 oxidation signal is attenuated by the CA film, concentrations of H_2O_2 in the picomolar range should still be detectable. This report lacks a critical experiment, being the determination of H_2O_2 (spiked) in serum, plasma, etc. The results are encouraging for those who wish to determine H_2O_2 in a complex biological sample. In addition, there are many other selective membranes that could be tested.