

## Mediated Microbiosensors

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Microglucose sensors using cylindrical microelectrodes of 2  $\mu\text{m}$  were fabricated and their characteristics were evaluated using 1,4-benzoquinone (BQ) as an electron mediator. The dependence of the response on both glucose concentration and BQ concentration was examined. The theory here was compared to the experimental results obtained from micro- and conventional glucose sensors. The response to glucose was investigated at various concentrations of BQ. As BQ concentration was increased, a higher current response was observed. The maximum calibration curve was obtained at a BQ concentration of greater than 20 mM. A linear relationship was observed up to a glucose concentration of approx 40 mM at 20 mM BQ. The 2- $\mu\text{m}$  glucose sensor was compared with the larger size sensors. The widest linear range was observed in the calibration curve of the 2- $\mu\text{m}$  glucose sensor. The response of the cylindrical microglucose sensor was theoretically analyzed. This indicated that the wide linear range of the calibration graph can be obtained with the glucose sensor having a high enzyme activity. The calculated results demonstrated that with the larger electrode size and increased relative catalytic activity, a wider linear range could be obtained for a calculated glucose calibration curve. However, the linearity was found to be less dependent on the electrode size than the relative catalytic reactivity.

GDH-based glucose sensors using cylindrical platinum electrodes of 25 and 2  $\mu\text{m}$  diameter were constructed. The glucose calibration curves of GDH/diaphorase coimmobilized and GDH immobilized electrodes were investigated. Much higher response could be obtained when diaphorase was coimmobilized in the enzyme membrane. This indicates that NADH oxidation by ferricyanide did not rapidly occur. The effect of oxygen on the GDH and diaphorase coimmobilized electrode was investigated. The GDH-based micro glucose sensor was found to be unaffected by dissolved oxygen concentration.

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We fabricated a glucose sensor using glucose dehydrogenase, which does not react with oxygen. In addition, we constructed an integrated biosensor to make a simultaneous determination of glucose and galactose. Since GDH does not react with oxygen, glucose and galactose could be determined separately even if GDH and galactose oxidase were mixed and coimmobilized on two neighboring electrodes on the same substrate. Galactose could be determined from oxygen consumption detected by the oxygen electrode, and glucose concentration was determined from both electrode responses.