0273-2289/93/4101-2—0073$03.00

Miniature Clark Oxygen Electrodes Using Anisotropic Etching of Silicon and Their Application to Biosensors

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The Clark oxygen electrode is an electrochemical device used in biosensor development, clinical analysis, and fermentation monitoring. However, a problem is that currently available oxygen electrodes are very expensive and difficult to miniaturize. A major objective of this study was to make an inexpensive miniature oxygen electrode.

The miniature oxygen electrode chip is 2×15 mm with a sensitive area 0.2×2 mm. We formed rectangular grooves by anisotropically etching a (100) silicon substrate, and made the cathode and anode on this area. We used a gold or silver cathode and a gold or Ag/AgCl anode. The sensitive area was delineated by a negative photoresist. The grooves were filled with electrolyte over which a gas-permeable membrane was directly formed.

To begin with, we used an electrolyte impregnated in gel. However, to improve fabrication, long-term storage stability, and tolerance to steam-sterilization, we also used a KCl electrolyte mixed with a polyvinylpyrroridone paste and a screen-printing technique. The membrane materials were a negative photoresist, silicone resins, and silicone rubbers. Water essential for oxygen reduction was incorporated in the electrolyte by sterilizing the completed oxygen electrode in steam.

The electrode produced a very clear response curve without appreciable noise. The 90% response time was 1.3 min on average. Residual current at zero oxygen concentration was about 10% that for the oxygen-saturated state. The response time depended on the anode materials. The response with a Ag/AgCl anode was much faster than with a gold anode. The residual current was also reduced significantly by replacing the gold anode with a Ag/AgCl anode. We obtained a calibration curve with fairly

This report was presented at the US/Japan Workshop on Microfabrication and Biosensors, July 21–24, 1992, sponsored by the National Science Foundation.

good linearity at 27°C. The oxygen electrode always survived at least one sterilization by steam.

A miniature glucose sensor was made using the oxygen electrode. The response time of the sensor was 60 s on average. Its calibration curve was linear for glucose concentrations from 60 μM to 1.1 mM. The sensor maintained almost the same response for about 1 mo.

A miniature L-lysine sensor was also made. To make the structure compact, autotropic bacteria were immobilized in the groove along with the KCl electrolyte. After the sensitive area was covered with a silicone resin gas-permeable membrane, L-lysine decarboxylase enzyme was immobilized on the sensitive area by bovine serum albumin and glutaraldehyde. The response was best at 33°C and pH 6.0. Under optimum conditions, the calibration curve was linear between 25 and 400 μM L-lysine and saturated above this concentration.

Such amino acid sensors were integrated on a chip. We used four oxygen electrodes as transducers that were stuck together back-to-back using a silicone resin. We used L-glutamate oxidase to detect L-glutamate, and three decarboxylases to detect L-lysine, L-arginine, and L-histidine. L-glutamate is measured by directly monitoring decrease of oxygen concentration. Detection of the other three sensors is based on the same principle as the previous L-lysine sensor. The glutamate sensor showed a weak dependence on pH between 5.5 and 8.5. The other sensors all had a maximum response at an optimum pH for each enzyme. The L-glutamate sensor showed weak dependence on temperature at temperatures from 31°C to 39°C. The other three sensors gave a maximum between 31°C and 33°C. Although they have different sensitivities, they all showed good linearity.