

Immobilization Techniques to Avoid Enzyme Loss from Oxidase-Based Biosensors: A One-Year Study

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Abstract

Background:

Continuous amperometric sensors that measure glucose or lactate require a stable sensitivity, and glutaraldehyde crosslinking has been used widely to avoid enzyme loss. Nonetheless, little data is published on the effectiveness of enzyme immobilization with glutaraldehyde.

Methods:

A combination of electrochemical testing and spectrophotometric assays was used to study the relationship between enzyme shedding and the fabrication procedure. In addition, we studied the relationship between the glutaraldehyde concentration and sensor performance over a period of one year.

Results:

The enzyme immobilization process by glutaraldehyde crosslinking to glucose oxidase appears to require at least 24-hours at room temperature to reach completion. In addition, excess free glucose oxidase can be removed by soaking sensors in purified water for 20 minutes. Even with the addition of these steps, however, it appears that there is some free glucose oxidase entrapped within the enzyme layer which contributes to a decline in sensitivity over time. Although it reduces the ultimate sensitivity (probably via a change in the enzyme's natural conformation), glutaraldehyde concentration in the enzyme layer can be increased in order to minimize this instability.

Conclusions:

After exposure of oxidase enzymes to glutaraldehyde, effective crosslinking requires a rinse step and a 24-hour incubation step. In order to minimize the loss of sensor sensitivity over time, the glutaraldehyde concentration can be increased.

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