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An amperometric biosensor for dopamine based on mushroom tissue

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A biosensor for dopamine was developed wherein mushroom tissue was coupled with an amperometric sensor for oxygen. The device monitored the decrease in the dissolved oxygen in the medium as a result of the oxidation of the analyte in the presence of an enzyme contained in mushroom. The response of the biosensor was linearly related to the concentration of dopamine. The thickeness of the tissue and the pH of the analyte medium affected the responses of the sensor.

Keywords: dopamine sensor, plant-tissue biosensor, amperometric sensor, mushroom biosensor

Biosensors provide a simple, sensitive and rapid strategy for the determination of complex organic compounds. These devices couple a chemically-selective biological system with a sensitive transduce to give an output signal which is related to the concentration of the analyte.

Various types of biological elements have been employed in biosensors, and immobilized enzymes have been the most widely used. However, recently, the use of plant tissues in biosensors has merited some considerable attention. Plant tissues contain naturally immobilized enzymes, and are therefore simpler to use, less expensive and relatively more stable than the isolated enzymes [1,2].

The concept of biosensing has been applied by several workers for the measurement of dopamine. Dopamine is one of the important biogenic amines functioning in the human body as a neurotransmitter. A number of plant tissue-based sensor has been reported for dopamine. Banana pulp [3,4] and eggplant [5] have been employed in these biosensors, together with an amperometric transduction system.

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In this paper, a dopamine sensor based on mushroom tissue is described. Mushroom contains polyphenol oxidase, which catalayzes the oxidation of dopamine. This oxidation results in the consumption of oxygen, which can be monitored through an amperometric sensor.

Experimental

Reagents

All solutions were prepared with analytic-grade reagents and distilled deionized water. Stock solutions (1×10^{-2} M) of dopamine hydrochloride (Sigma) were prepared daily. Buffer solutions were prepared from potassium dihydrogen phosphate solution (0.05 M) and sodium hydroxide (0.05 M). The mushrooms were purchased from a local supermarket and stored at 4°C until use.

Instrumentation

The instrumentation system is diagrammed in Figure 1. It consists of a constant potential source, a current-to-voltage converter and a recorder. The constant potential source and the current-to-voltage converter were constructed based on an operational amplifier (UA 741). The constant potential source provide a voltage of -667 mV.

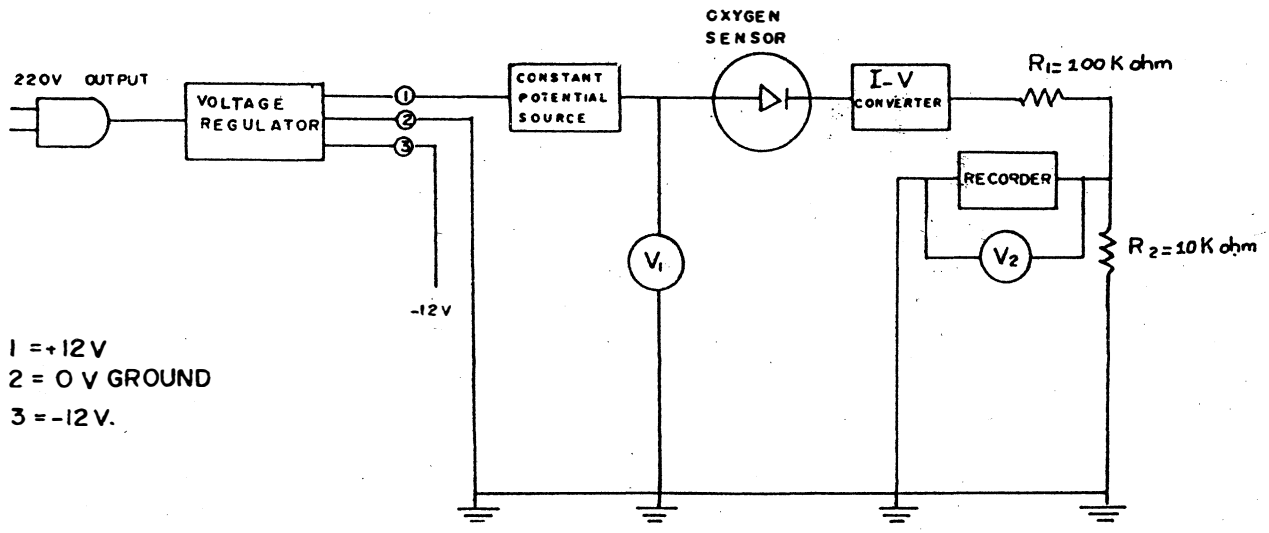

Figure 1. Block diagram of the circuit for the oxygen sensor

Sensor construction

The dopamine biosensor is essentially a fabricated Clark cell with a mushroom tissue attached to its sensing end (Figure 2). The cathode was a gold wire (diameter 1.02 mm) which was wound tightly into a flat coil of diameter 9.14 mm. The anode was a silver wire (diameter = 1.9 mm) which was formed into a helix around a plastic tube of 3 mm diameter and anodized to coat it with silver chloride. The electrolyte was a solution of potassium chloride (2.3 M) and a phosphate buffer (ph 7.3). The oxygen-permeable membrane was a polyethylene sheet with a thickness of about 4 mμ.

A slice of the mushroom tissue (thickness = 0.3 mm) was placed over the membrane of the Clark cell and enclosed by a dialysis membrane which was held in place by a silicone O-ring.

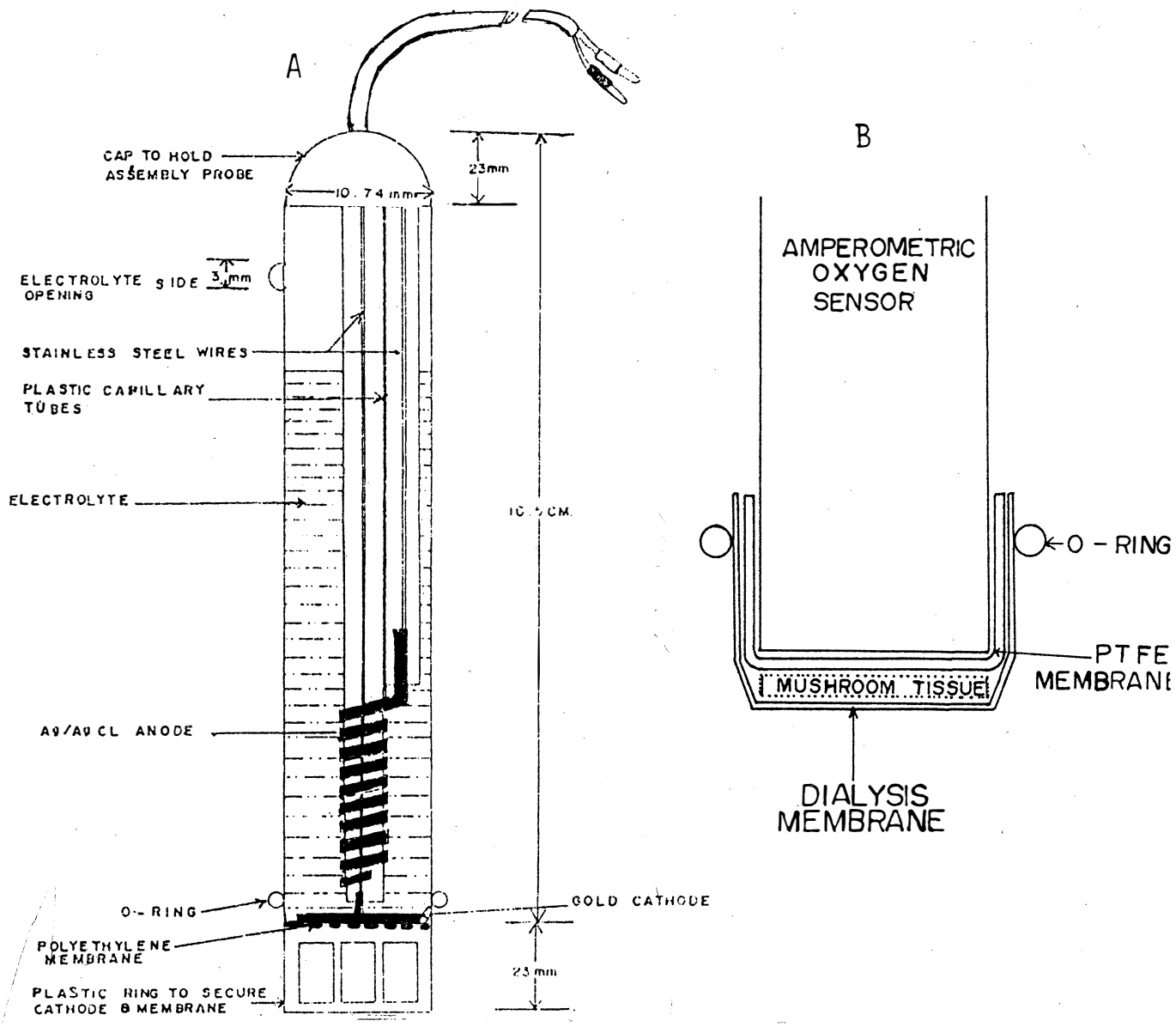

Figure 2. (A) Diagram of the oxygen sensor. (B). Diagram of the dopamine biosensor.

Measurements

The measurement were done in a small glass vessel containing 30 ml of phosphate buffer which was saturated with air. The sensor was immersed in buffer solution, and the steady state value of the response was recorded. A measured amount of the dopamine stock solution was then introduced into the buffer solution, and the response of the sensor was recorded until a steady state was attained.

Results and Discussion

Four different types of mushrooms were tried in this study: Agaricus biosporus, Catharellus, Boletus and Lepiota. Among these specimens, the Agaricus or the common button mushroom produced the largest response to dopamine, when it was coupled to an oxygen sensor. It also exhibited the fastest response. As a result, the succeeding measurements were carried out using this species.

Figure 3 shows the time response of the fabricated dopamine sensor. A decrease in current occurred, indicating a consumption of oxygen due to the oxidation of dopamine in the presence of the polyphenol oxidase in the mushroom tissue. A steady state response was attained within five minutes for a 3×10^{-4} M solution of dopamine. The reproducibility of this response was satisfactory, a relative standard deviation of 7.9% being obtained for a series of 11 successive measurements.

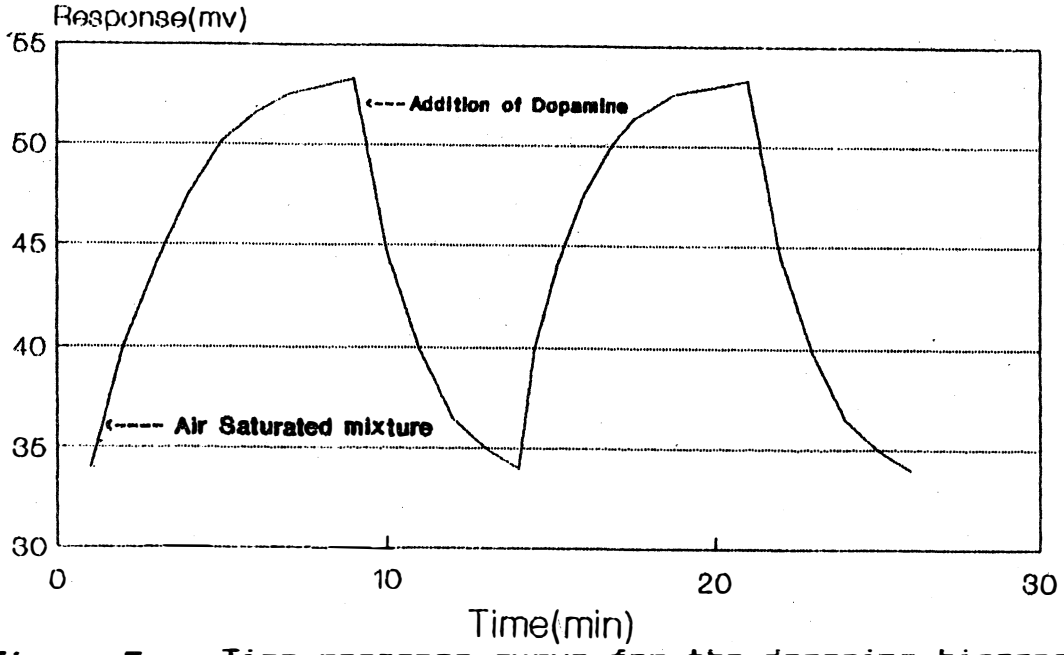

Figure 3. Time response curve for the depomine biosensor

The response of the sensor was affected by the thickness of the mushroom tissue used. Figure 4 shows the time response of sensors with different thickess of the tissue. The thicker tissues exhibited a lower and a slower response compared to the thinner tissues. This behavior can be attributed to the significance of the diffusion of the analyte through the tissue in the performance of the sensor. In view of these observations, a tissue thickness of 0.3 mm was adopted. Thinner tissues could not be used, because of their lack of mechanical stability.

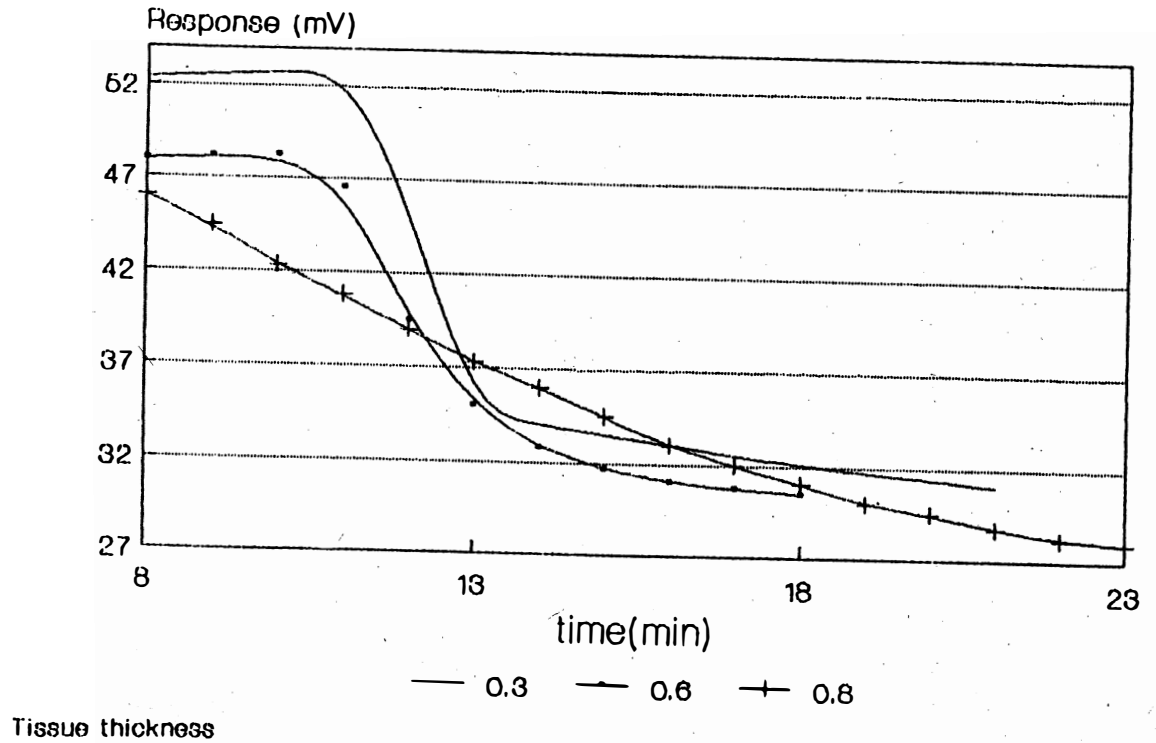

Figure 4. Response curve of dopamine biosensor with different thickeness of mushroom tissue.

The response of the sensor was not affected significantly by the pH of the analyte solution within the range of pH 6 to 8 (Figure 5). Measurements were carried out at a pH of 7.4.

The variation of the sensor response with the concentration of dopamine is illustrated in Figure 6. A linear correlation (Pearson correlation coefficient, -0.9997) was observed for dopamine concentrations in the range of 0.1 to 1.2 mM dopamine. The sensitivity of the sensor can be assessed from the slope of the calibration curve, and was determined to be 1.97 mV/mM dopamine

The lifetime of the sensor was seven days when it was stored in a phosphate buffer at 4°C. The response of the sensor decreased by than 50% after the tenth day.

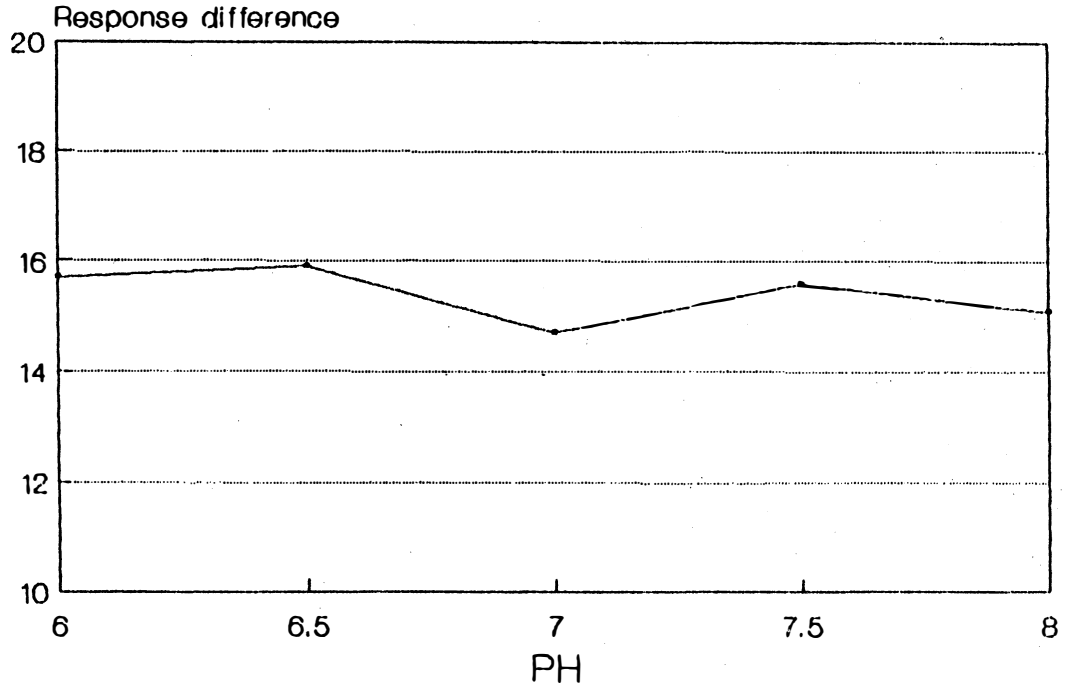

Figure 5. Effect of pH on the response of the dopamine sensor.

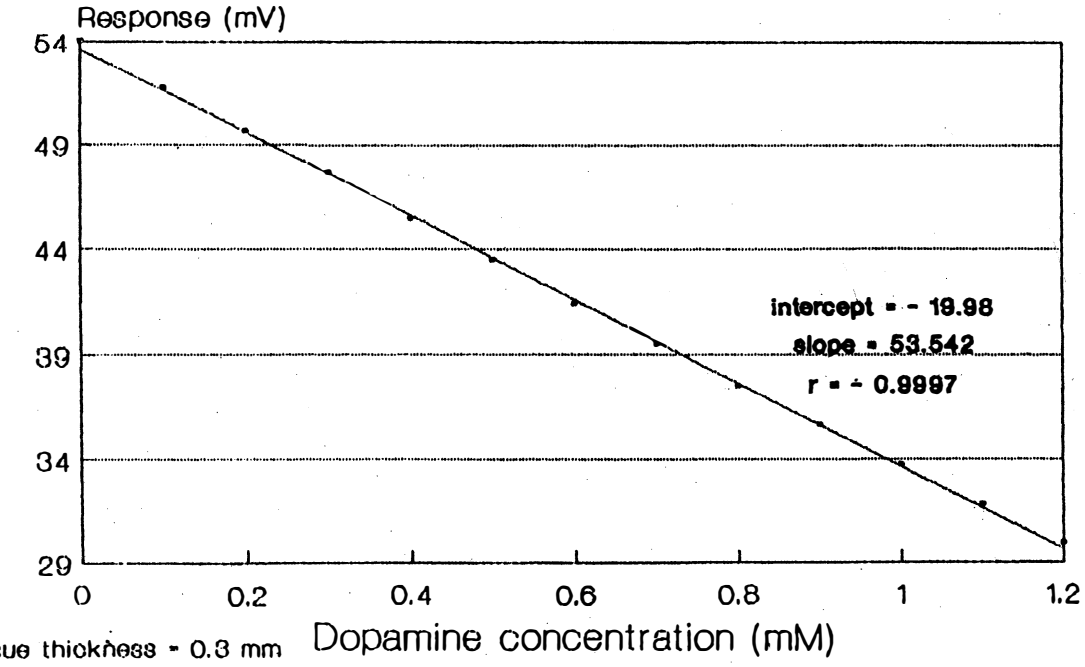

Figure 6. Calibration curve for the dopamine biosensor.

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