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# A Novel Catechol Oxidase Enzyme Electrode for the Specific Determination of Catechol

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**An enzyme electrode for the specific determination of catechol was developed by using catechol oxidase (EC 1.10.3.1) from eggplant (*Solanum melangena* L.) in combination with a dissolved oxygen probe. Optimization studies of the prepared catechol oxidase enzyme electrode established a phosphate buffer 50 mM at pH 7.0 and 35°C to provide the optimum conditions for affirmative electrode response. The enzyme electrode response depended linearly on a catechol concentration range of $5 \cdot 10^{-7}$–$30 \cdot 10^{-5}$ M with a response time of 25 sec and substrate specificity of the catechol oxidase electrode of 100%. The biosensor retained its enzyme activity for at least 70 days.**

**Key words:** enzyme electrode; biosensor; catechol; eggplant (*Solanum melangena* L.)

## Introduction

Catechol has important uses in the manufacture of dyes, rubbers and drugs[1] and it acts as an antioxidant for lubricating oils and photographic processings and as an agent for oxygen removal. In addition, catechol is also found in physiological fluids such as urine,[2] in certain foods, and in cigarette smoke.[3] Catechol determination is especially important for the analysis of soil pollution[4] and for research into the carcinogenic effects of catechol and its derivatives that are formed during the metabolic process of humans.[5,6]

The determination methods for catechol are generally based on the color reaction of a phenolic substance with a number of oxidants and complex-forming compounds.[7] None of these methods is specific for catechol and suffers from interference by the other phenolic compounds. The analysis method thus requires a preceding separation from other phenolic substances usually occurring alongside catechol. This paper describes a sensitive, simple, rapid and economic method for determining catechol by means of an oxygen probe coated with immobilized catechol oxidase ((catechol:oxygen oxidoreductase) (EC 1.10.3.1)) isolated from eggplant (*Solanum melangena* L). The enzyme specifically oxidizes catechol to an *o*-quinone by incorporating 0.5 mol oxygen.

To prepare the catechol oxidase enzyme electrode, the enzyme was immobilized on the surface of a YSI 5739 oxygen probe by crosslinking with gelatine with the help of glutaraldehyde. The prepared enzyme electrode could then be used for the determination of catechol, as the amount of consumed oxygen, which was determined by a YSI 54 A oxygen meter according to the reaction shown next, was proportional to the catechol concentration in the reaction medium

$$\text{Catechol} + 1/2\,O_2 \xrightarrow{\text{Catechol oxidase}} o\text{-Quinone} + H_2O.$$

## Materials and Methods

*Reagents.* Catechol oxidase (EC 1.10.3.1) was isolated from eggplant (*Solanum melangena* L.) which had been purchased from a local market and purified by using conventional separation techniques. Calf skin gelatin (225 bloom) and glutaraldehyde were obtained from Sigma Chem. Co. (St. Louis, U.S.A.). All other chemicals being purchased from E. Merck (Darmstadt, Germany).

*Apparatus.* A YSI 54 A oxygenmeter and YSI 5739 dissolved oxygen (DO) probe (YSI Co., Yellow Springs, Ohio, U.S.A.) were used. A dissolved oxygen concentration change could be determined by the measurement system as low as 0.025 mg/l. All the measurements at constant temperature were taken while using an Ultrathermostat (Colora, Germany).

*Enzyme isolation and purification.* The eggplants were homogenized in two times their weight of a 0.1 M potassium phosphate buffer at pH 6.5 in a Braun blender for 2 min. and, after being filtered through cheese cloth, the solution was centrifuged at $10{,}000 \times g$ for 15 min. The filtrate served as the source of the crude extract. Ammonium sulphate was added to the crude extract to give 70% saturation. The precipitate was collected by centrifugation and redissolved in a phosphate buffer (0.1 M at pH 7.0), and the resulting solution was dialyzed against several changes of the same buffer overnight. The dialysed fraction was then loaded into a $2.6 \times 40.0$-cm column of DEAE Sephadex that had been equilibrated with the 0.1 M phosphate buffer at pH 7.0. After washing the column with the same buffer, the bound proteins were eluted stepwise with 40, 80, 200 and 500 mM KCl, respectively. The active fractions were pooled, concentrated by ultrafiltration, and applied to a $1 \times 70.0$-cm column of Sephadex G-200. The active fractions were kept for SDS-polyacrylamide gel electropho-

*Abbreviation*: DO, dissolved oxygen

resis (12.5%), molecular weight determination by gel filtration, and determination of some properties of eggplant catechol oxidase. Enzyme activity was measured by determining the amount of quinone which reacted with proline to produce a red compound.[8] The optimum pH value for preparing the catechol oxidase from eggplant was determined as 7.0, the optimum temperature was 30°C. The $K_m$ value for catechol was $1.1 \times 10^{-4}$ M, molecular weight of the enzyme was evaluated as 120 kDa.

*Electrode preparation.* The gas-selective teflon membrane of a YSI 5739 (DO) probe was pretreated with a 0.5% lauryl sulphate solution in a 50 mM phosphate buffer at pH 7.5. Catechol oxidase (5 mg) and 225 bloom gelatin (10 mg) were mixed at 38°C in 300 $\mu$l of the phosphate buffer (pH 7.5). The mixed solution (200 $\mu$l) was spread over the DO probe membrane immediately and allowed to dry at 4°C for one hour. Finally, it was immersed in 1.25% glutaraldehyde in the 50 mM phosphate buffer at pH 7.5 for 5 min. The enzyme electrode had 0.15 IU/cm$^2$ of catechol oxidase activity.

*Measurement of the oxygen consumption by the catechol oxidase enzyme electrode.* The measurements were carried out at 35°C with various catechol concentrations under steady state conditions. All the measurements were carried out by using a thermostatic cell,[9] phosphate (50 mM at pH 7.5) being used as the working buffer. With the steady state method, the addition of catechol caused a rapid current decrease due to oxygen consumption in the immobilized tissue layer which reached a steady state within 25 sec, the dissolved oxygen concentration then being recorded by an oxygen meter.

## Results and Discussion

*Enzyme electrode optimization*

*Effect of pH.* The pH dependence of the electrode signal was investigated. The optimum pH value was found to be 7.0 (Fig. 1).

*Effect of temperature.* The effect of varying the assay temperature (30–40°C) was examined (Fig. 2). The electrode response was unaffected by temperature up to 35°C, but exhibited a decrease with any further increase in temperature, thereby suggesting 35°C as the optimum working temperature.

*Effect of the enzyme concentration.* The enzymatic activity of the bioactive membrane layer depended upon the amount of the enzyme. The effect of varying the concentration of the enzyme ($7.5 \cdot 10^{-2}$ IUcm$^{-2}$, $15 \cdot 10^{-2}$ IUcm$^{-2}$ and $22.5 \cdot 10^{-2}$ IUcm$^{-2}$) on the electrode response was examined to identify chose the best electrode response, the highest response being observed at $22.5 \cdot 10^{-2}$ IUcm$^{-2}$. The electrode response decreased with decreasing in enzymatic activity. An amount of enzyme providing $15 \cdot 10^{-2}$ IUcm$^{-2}$ of catechol oxidase activity was preferable to ensure high stability of the bioactive membrane layer.

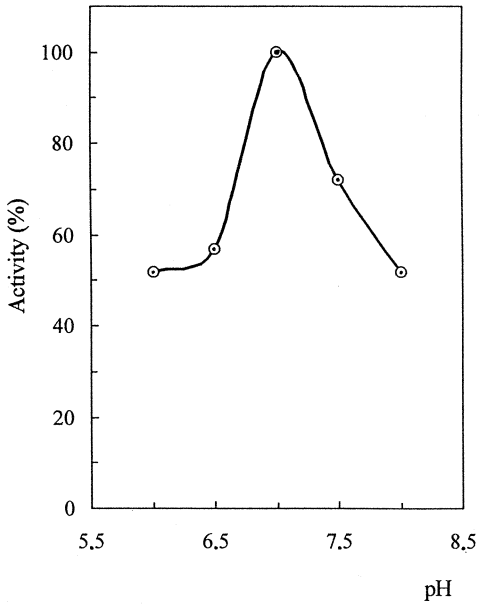


**Fig. 1.** Effect of pH value on the Electrode Response (50 mM phosphate buffer at 35°C).

The substrate concentration was $20 \times 10^{-5}$ M, and the amounts catechol oxidase, gelatin and glutaraldehyde were kept constant at $15 \times 10^{-2}$ IU/cm$^2$, 5.9 mg of gelatin/cm$^2$ and 1.25%, respectively.

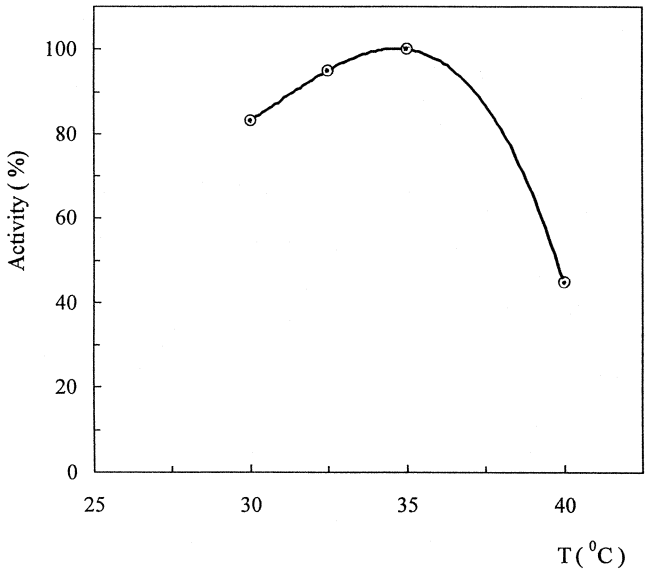


**Fig. 2.** Effect of Temperature on the Electrode Response (50 mM phosphate buffer at pH 7.0).

The substrate concentration was $20 \times 10^{-5}$ M, and the amounts of catechol oxidase, gelatin and glutaraldehyde were kept constant at $15 \times 10^{-2}$ IU/cm$^2$, 5.9 mg of gelatin/cm$^2$ and 1.25%, respectively.

*Effect of the gelatin concentration.* The effect of varying the concentration of gelatin (2.95 mg·cm$^{-2}$, 5.90 mg·cm$^{-2}$ and 7.85 mg·cm$^{-2}$) on the electrode response was investigated. The electrode response increased as the concentration of gelatin in the bioactive membrane layer was decreased. However, a moderate level of gelatin such as 5.9 mg·cm$^{-2}$ resulted in better

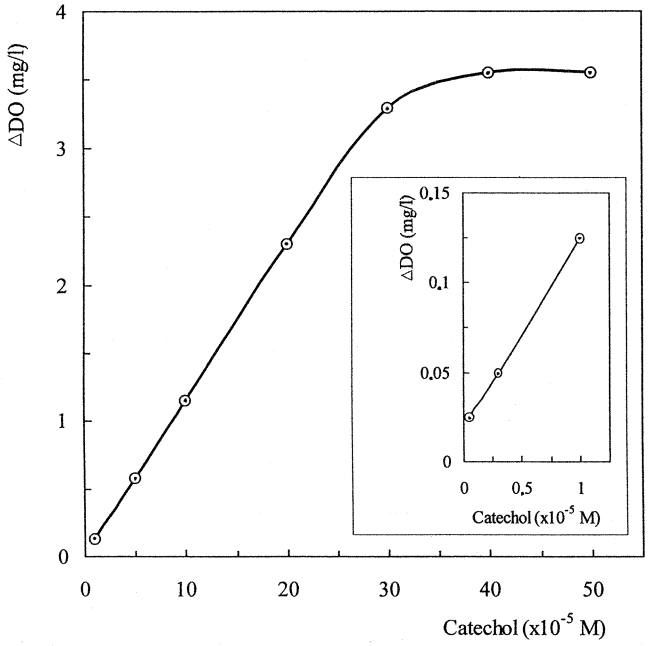


**Fig. 3.** Standard Curve of the Catechol Oxidase Enzyme Electrode (50 mM phosphate buffer at 35°C).

The amounts of catechol oxidase, gelatin and glutaraldehyde were kept constant at $15 \times 10^{-2}$ IU/cm$^2$, 5.9 mg of gelatin/cm$^2$ and 1.25%, respectively.

membrane stability than with 2.95 mg·cm$^{-2}$, although the electrode response was higher at the lower gelatin concentrations. Therefore, 5.9 mg·cm$^{-2}$ of gelatin was preferable during the work.

*Effect of the glutaraldehyde concentration.* The effect of varying the concentration of cross-linking reagent glutaraldehyde (1.25%, 2.5% and 5.0%) on the electrode response was examined. The electrode response was found to decrease with increasing glutaraldehyde concentration due to the limited diffusion of the substrate into highly cross-linked membrane layer.

*Analytical Characteristics*

*Linear range.* A typical calibration curve for the catechol oxidase enzyme electrode includes a linear range from $5 \cdot 10^{-7}$–$30 \cdot 10^{-5}$ M catechol (Fig. 3).

*Selectivity.* The selectivity of the electrode was evaluated by measuring the response to compounds which may cause possible interference. Of the various substrates tested, *i.e.*, catechol, adrenalin, phenol, hydroquinon, L-ascorbic acid and 8-hydroxyquinoline, only catechol was found to be selective with a relative activity of 100%.

*Accuracy.* The enzyme electrode was tested for its accuracy on ten standard solutions of catechol, each containing $10 \cdot 10^{-5}$ M, and gave $9.98 \cdot 10^{-5}$ M with a $\pm 0.25 \cdot 10^{-5}$ standard deviation and 2.48% variation coefficient as the mean catechol concentration.

*Stability.* A thermal stability investigation showed no activity loss for the biosensor after being held for 27 hours under working conditions (50 mM phosphate buffer at pH 7.0 and 35°C), this period being sufficient for performing more than 150 assays. When stored at 4°C, it retained more than 90% of its activity for 70 days.

Catechol oxidase was immobilized by covalent bonding in gelatin to form a bioactive membrane layer which was directly cast on the teflon membrane of a dissolved oxygen probe. The catechol oxidase enzyme electrode compared earlier catechol sensors and it was found that the new method was specific for catechol, economic and used conventional apparatus. There was no response to other similar compounds tested. This very high degree of specificity is comparable to that of another biosensor for catechol that has previously been reported.[10,11] The major advantage compared with the original electrode described by Navaratne, Lin and Rechnitz[11] lies in increased linear range from $5.0 \cdot 10^{-6}$–$4.5 \cdot 10^{-5}$ M catechol to $5 \cdot 10^{-7}$–$30 \cdot 10^{-5}$ M. The catechol biosensors described by Rechnitz[11] and Uchiyama *et al.*[10] Reportedly had lives of 23 days and 18 days, respectively, while, the life of our sensor was at least 70 days.

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