



LACCASE BIOSENSORS BASED ON MERCURY THIN FILM ELECTRODE

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 **Electrochemical biosensor can be explained as a molecular sensing device that combines a biological recognition element to an electrode transducer.**

 **The success of an electrochemical sensing process relies mainly on a proper choice of the working electrode. Gold, platinum, carbon based and mercury thin film electrode (MTFE) were utilized as electrode transducers .**

Mercury thin film electrodes

-  consist of a very thin film (10-100 μm) layer of mercury that is distributed over the support material like glassy carbon electrode (GCE)
-  are composed of many droplets that lead to a lower hydrogen over voltage.

-  **provide larger surface to volume ratio and can be utilized in different cell configurations.**
-  **as small quantities of mercury is used for the preparation of MTFE, the consumption of metallic mercury is minimized.**

SPGEs

- are based on screen-printing technology
- can be employed as low-cost disposable electrochemical sensors.

The determination of phenolic compounds is of great importance

They are widely used in industrial processes, such as the manufacture of plastics, polymers, drugs and dyes.

These compounds belong to a class of polluting chemicals that are easily absorbed by animals and humans through the skin and mucous membranes.

Their toxicity affect a great variety of organs and tissues.



The development of procedures for detection and simultaneous determination of phenolic compounds in different matrices is highly desired.

- **Biosensors using laccase as detection element have been developed to detect phenols in effluents.**
- **In fact, laccases (benzendiol: oxygen oxidoreductases; EC 1.10.3.2), which are multi-copper enzymes widely distributed in plant and fungal species, oxidize phenols where molecular oxygen is the terminal electron acceptor of the oxidation process.**

Present study includes the examination of MTFE as the part of laccase biosensor for the detection of phenolic compounds where GCE and SPGE were used as the support material for film formation.

MATERIAL AND METHODS

Synthetically concocted waste water composition; 50 g/L NaCl and 100 g/L phenol in 1.0 M HCl solution.

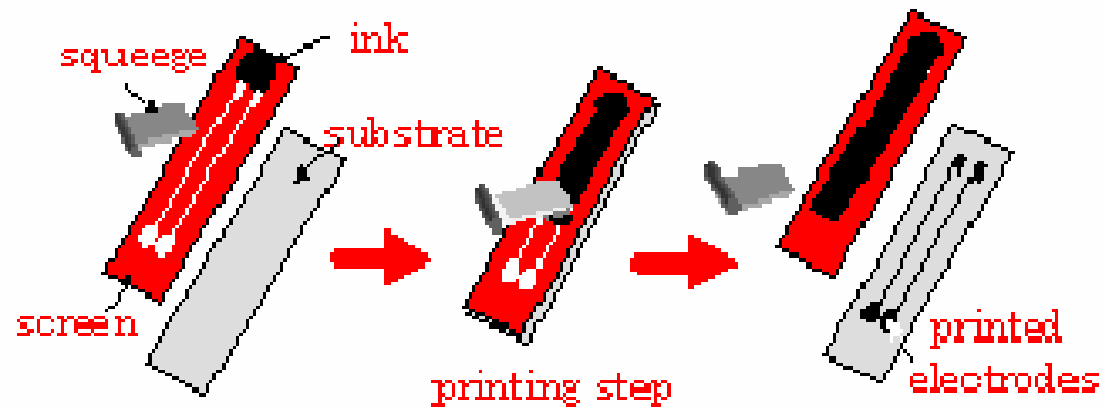
Screen Printed Electrodes (SPE); carbon ink (Du Pont 7101) was used for printing working electrodes on a PVC substrate.

The conducting paths and pads were deposited directly on the PVC sheets using Ag/Pd ink (DuPont, 5025).

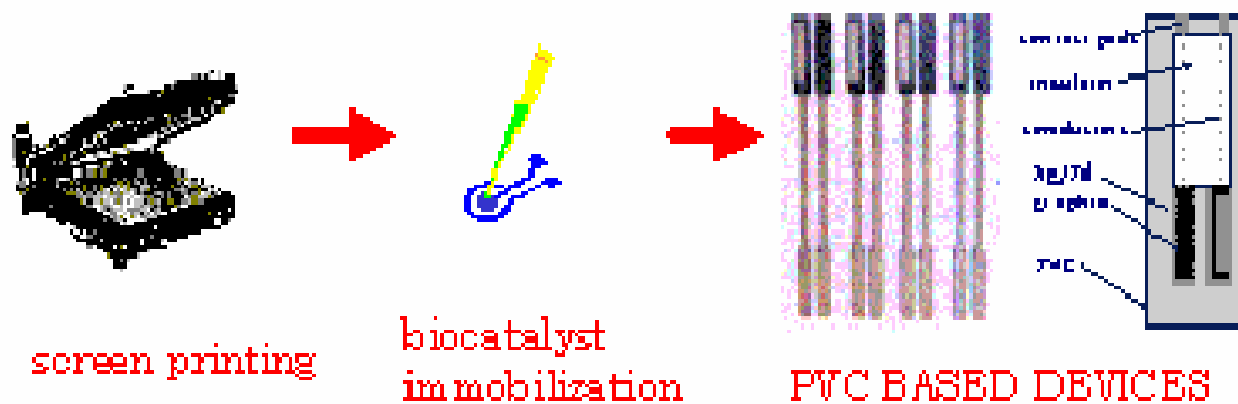
Ag/AgCl ink was deposited to obtain the reference electrode.

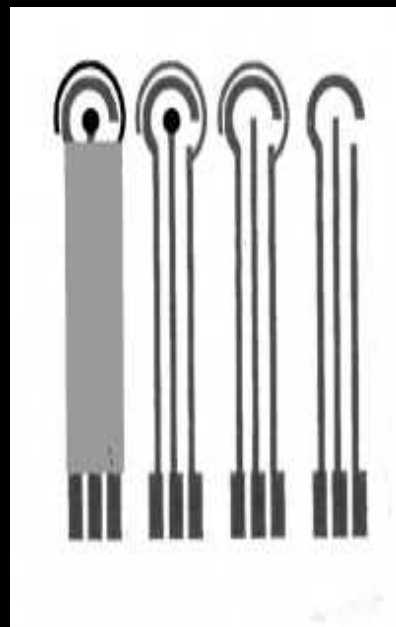
After each printing step, the paths were treated at 60°C for 60 min.

A. SCREEN PRINTING



B. PVC DEVICES





(d) (c) (b) (a)

**SPGEs; a)Ag/Pd counter electrode,
conductive pads, b)Ag/AgCl reference
electrode, c)carbon based working
electrodes, d) insulator**

Biological Material

Laccase was isolated from the culture filtrates of the white-rot fungus *Trametes versicolor* (ATCC 11 235).

T. versicolor was maintained at 4°C on 2% malt agar and grown in 100 ml malt extract broth (2%) for three days in a nitrogen-limited medium consisting of 10 g glucose, 1 g $\text{NH}_4\text{H}_2\text{PO}_4$, 0.05 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g CaCl_2 and 0.025 g yeast extract.

The cultures of *T.versicolor* were incubated at 26°C on a rotary shaker at 175 rpm. After 72 h cultivation, concentrated solution of phenol was added to the cultures to give 10 mg/l.

Laccase activity was estimated by oxidation of ABTS.



Apparatus

- **Chronoamperometric measurements were performed using a PALM SENS electrochemical measurement system from PALM Instruments B.V. (Netherlands).**
- **DP-voltammograms were obtained with a Metrohm 693 VA TraceAnalyzer and a 694 VA Stand with Ag/AgCl reference and Pt auxiliary electrodes.**

Electrode Preparation



MTF was formed on glassy carbon (Type I) and screen printed graphite electrode (Type II) .


MTF electrode was prepared ex-situ by introducing 20 ml of mercury plating solution containing 200 mg / ml Hg (II) ions in 1.0 M HCl solution into the cell. A deposition potential of -800 mV was applied for 5 min after 5 min deaeration of the solution.

In case of using SPG electrode (diameter of 3mm), MTF was deposited on the graphite working electrode by using the same procedure as described above.



Laccase and 225 bloom gelatin (10 mg) were mixed at 38 °C in potassium phosphate buffer, pH 7.0 (250 μ l).


50 μ l of mixed solution was spread over the electrode surface and allowed to dry at 4 °C for 1 h.


 The electrode was immersed in 2.5 % glutaraldehyde in 50 mM phosphate buffer (pH 7.0) for 5 min. Moreover, the procedure belong to SPGE was the same as GCE, except 5 μ l of mixed solution was placed on graphite working electrode.

 Both types of electrode include 7.0 unit of laccase activity.

Measurements

- **Dp voltammograms were recorded between -100 and -1200 mV with a 50 mV pulse amplitude. Measuring time was 40 ms and the current was sampled in 20 ms.**
- **All other measurements were done at 25°C under continuous and constant magnetic stirring and varying substrate concentrations in steady-state conditions in 50 mM oxygen saturated acetate buffer (pH 4.5) without any deaeration.**

 The working electrode was polarized at -0.3 V vs Ag/AgCl electrode. The duration of each analysis was 200 sec. The residual current was allowed to decay in the presence of working buffer before the addition of substrate solution

 50 mM of acetate buffer, pH 4.5 was used as working buffer for biosensor. All the optimization studies were performed with Type I, while both Type I and Type II electrodes were analytically characterized.

RESULTS AND DISCUSSION

Developed biosensor provides the detection of phenolic compounds by monitoring the consumption of dissolved oxygen.

In order to observe the reduction peaks of oxygen hence explore the optimum operating potential, voltammograms were recorded by using MTFE in the presence of 50 mM acetate buffer (pH 4.5).

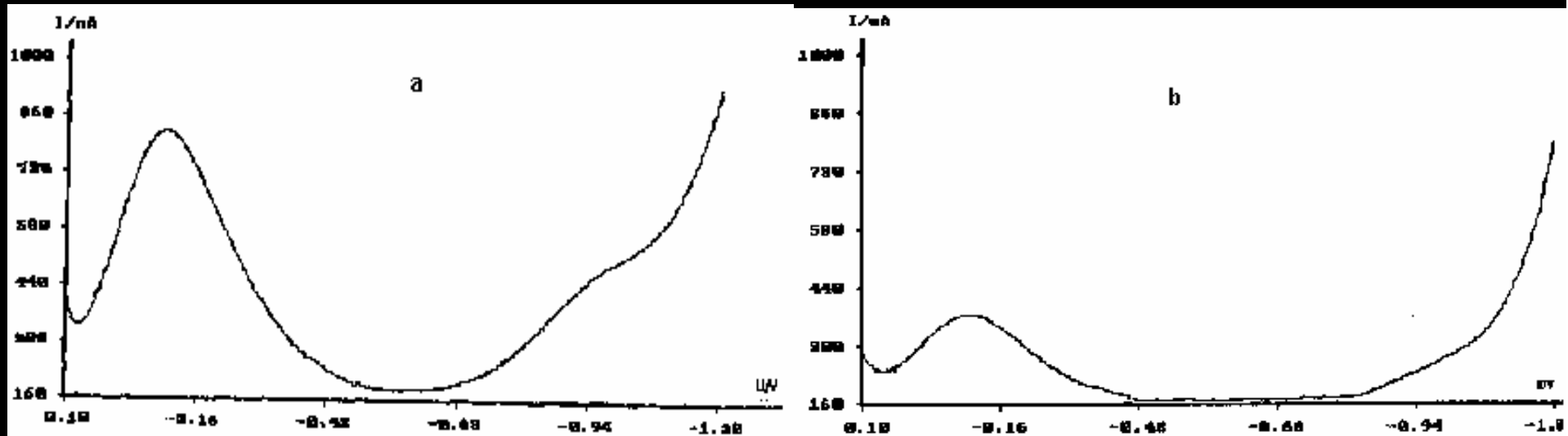


Figure 1. The dp voltammograms of oxygen reduction obtained with MTFE in the presence of 50 mM acetate buffer (pH 4.5) (a) without any deaeration (b) after 5 min. N_2 deaeration.

The operating potential of reduction of oxygen to hydrogen peroxide, was chosen as -300 mV v.s Ag/AgCl and used for further studies.

Enzyme electrode optimization

pH effect

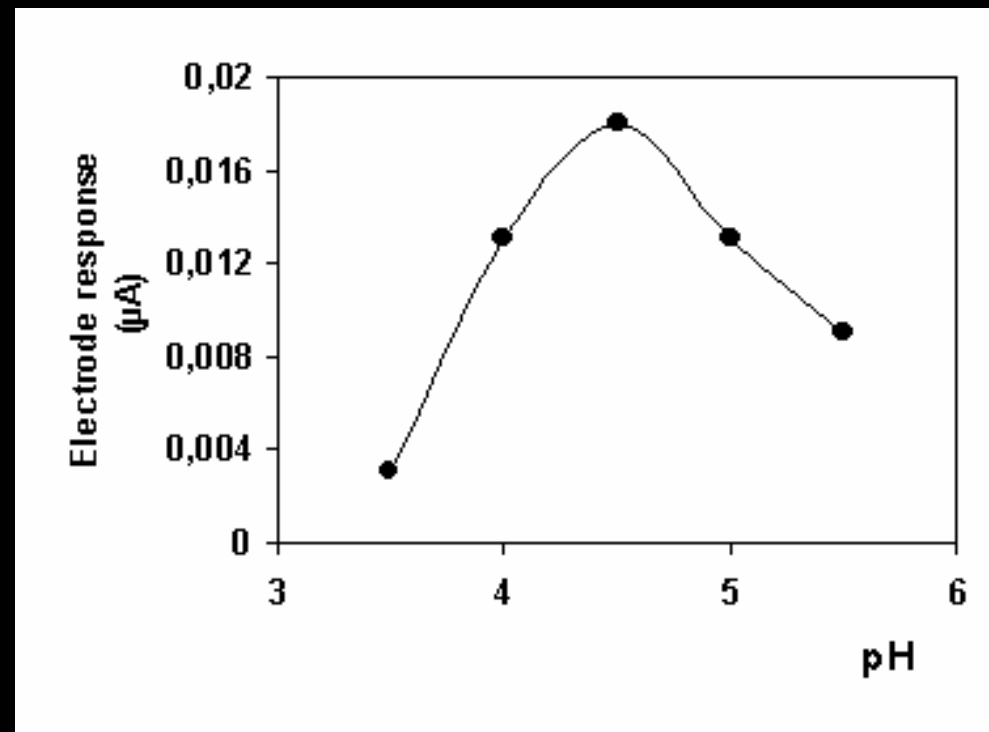


Figure 2. Optimum pH of laccase biosensor (pH 3.5-5.5; acetate buffer, T;25°C).

The maximum response was observed in acetate buffer (50 mM) at pH 4.5.

Effect of temperature

- The effect of temperature on the response of the biosensor system was also searched and maximum sensor response was found at 35°C.
- Further experiments were performed at 25°C in order to avoid activity loss.

Analytical Characteristics

Linear range

Catechol with Type I biosensor the linear concentration range is between 5.0×10^{-7} - 5.0×10^{-6} M with the equation of $y=0.0054x+0.0035$, where $r^2=0.990$.

Phenol with Type I biosensor the linear concentration range is between 2.5×10^{-7} - 2.0×10^{-6} M with the equation of $y=0.0272x+0.003$, where $r^2=0.997$.

Catechol with Type II biosensor, the linear concentration range is between 2.5×10^{-6} - 3.0×10^{-5} M with the equation of $y=0.0051x-0.0045$ where $r^2=0.990$.

Table 1. Linear regression coefficients, Standard error (S.E), coefficient of variation (cv), Lod and Loq of the biosensors obtained by MTF deposited on GCE and SPE

Electrode	Surface (mm ²)	Substrate	Slope		CV (%)	Sensitivity ^a nA.μM ⁻¹ mm ⁻²	Intercept		Lod ^b (μM)	Loq ^b (μM)
			nA.μM ⁻¹	S.E			(μA)	S.E		
Type I	12.6	Phenol	5.4	±0.3	5.5	0.4	3.5	±0.8	0.41	0.75
		Catechol	27.2	±1.1	4.0	2.2	3.0	±0.8	0.10	0.18
Type II	7.1	Catechol	27.0	±1.0	3.7	3.8	3.0	±0.7	2.00	3.00

^aCalculated as sensitivity= (slope) × (electrode surface)⁻¹

^bLimit of detection and limit of quantitation have been calculated with the graphical method from Meier and Zund using interval curves at confidence=95%.

Sample Application

Developed sensor was applied for the determination of phenol in waste water samples.

When 0.25 and 0.5 μM of phenol included synthetic waste water samples were added the concentrations were calculated as 0.25 ± 0.021 and 0.51 ± 0.005 μM from the calibration curve.

It could be concluded that the system could be easily and usefully addressed for the screening of phenolic compounds in industrial waste water samples with acidic nature.

Conclusion

- **Developed system allows the usage of mercury electrodes in biosensing systems.**
- **On the other hand, the attractive behavior of thin film mercury SPGEs as biosensor compound, have proven that these electrodes perform in a manner comparable with conventional electrodes for these systems.**



Bi-film electrodes have recently been introduced as an alternative electrode material to MTFE. The attractive stripping performance of this new electrode material is demonstrated to be comparable to that of mercury ones. Besides, BIFE electrodes provide ease in the in situ deposition, high sensitivity and well defined current peaks can be given as the other important analytical characteristics.



BIFE;

Potential window; -200 and -1200 mV

**Working condions; pH 4.5 acetate
buffer**

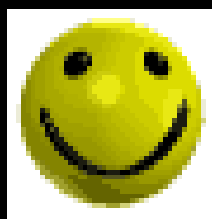
Phenol to quinon?

As a part of Laccase biosensor



MTFEs

The proposed system could be functional as disposable arrays for field use. The immobilization method provides the mild conditions to continue the enzyme activity. Utility of gelatin as an immobilization material provides the use of same electrode for several time without needing any film formation.



The advantages of both screen printed disposable electrodes and MTFE enable us to get economical and easy to use systems for environmental analyses.

