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Biosensing of Catechol by Screen-Printed Electrode Using Potato and Banana Extract

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Abstract

Catechol or Pyrocatechol is a hazardous chemical found in the environment, released during its manufacture and use. This paper presents electrochemical biosensing of catechol using crude sources of enzyme catechol oxidase. Catechol is oxidized to brown colored benzoquinone in presence of the enzyme catechol oxidase. We extracted the enzyme from the cells of freshly cut banana and potato. The freshly prepared extract of banana/ potato was immobilized in a polymeric porous network over a screen-printed working electrode and the rate of oxidation of catechol was measured amperometrically. The response was linear in nature and varied in the range of 0-10 ppm.

Keywords: *Catechol, biosensor, potato extract, polymer electrode, amperometry*

1.Introduction

The deleterious effect of phenolic compounds is well known to the environmentalists so the quantitative detection of such compounds is essential [1]. Mono and polyphenols are released in the environment as the by-product of paper pulp industries and the breakdown products from pesticides [2]. The conventional methods of measurement of phenolic compounds are colorimetry, gas chromatography, liquid chromatography and capillary electrophoresis [2]-[4]. However except colorimetric method, these techniques are expensive, time consuming, needs sample pre treatment and unsuitable for on site or in field monitoring [5]. The colorimetric method suffers from interference due to non-specificity.

Catechol, a substituted phenol is a hazardous chemical found in the environment, released during its manufacture and use. Through the ingestion of contaminated food and polluted drinking water, catechol is exposed to human and other living beings. This harmful chemical causes acute toxicity by depressing central nervous system of animals, rising blood pressure, creating dermatitis and other skin diseases and in extreme cases, respiratory failure may lead to death. A colorless solution of catechol is oxidized to brown colored benzoquinone in presence of the enzyme catechol oxidase or polyphenoloxidase or tyrosinase. Polyphenol oxidases (PPOs EC. 1.14.18.1) are a group of copper proteins that are widely distributed from bacteria to mammals [6]. They catalyze two reactions: the hydroxylation of monophenols to o-diphenols and the oxidation of diphenols to o-quinones. Enzymatic browning is the main function of PPOs in fruits and vegetables, causing unpleasant sensory odour and losses in nutritional quality [7]. Polyphenol oxidase can be extracted from the cells of freshly cut banana, potato, mushroom, apple, quince, and spinach [8]-[11]. Using crude potato extract the amount of polyphenols in wastewater could be determined spectrophotomertrically [12]. But this method suffers from certain limitations like interference, sample pretreatment, lengthy procedure and also it is not suitable for in-field testing.

Biosensor, on the other hand, is based on bio recognition material e.g. enzyme and a physicochemical transducer and may overcome such limitations. There are various biosensors reported in the literature [12]-[15] for the detection of catechol using PPO or tyrosinase. However, these are based on commercial grade enzymes and hence costly. Some researchers [14] extracted the enzyme from plant source Amorphophallus companulatus and immobilized in an electrochemically etched surface of p-type silicon [14]. The principle of this sensor was based on the change in the conductivity of the tyrosinase-entrapped porous silicon matrix which was proportional to the analyte concentration. The system was however very complex to fabricate. The biosensor based on immobilization of commercial tyrosinase, laccase and peroxidase have been optimized for catechol determination, tested in a flow injection system for amperometric monitoring of a large number of phenolic compounds where the limit of detection for phenol was in the micro molar range [15]. In this work, a screen-printed electrode (SPE) was utilized to motor electrochemical behavior of catechol by cyclic voltammetric techniques followed by amperometry. The modified graphite working electrode of the SPE gave oxidation peak current in the linear range from $1x10^{-6}$ to $1x10^{-4}$ M [16].

Freshly prepared potato and banana extract was used as the enzyme source. This extract was immobilized in a polymeric porous network on a screen-printed electrode and the rate of oxidation of catechol was measured amperometrically.

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Experimental

2.1Materials and Reagents

In this study, buffer (Sigma) of 0.1(M) Na_2HPO_4-NaH_2PO_4 (pH=6.8) containing 0.1(M) KCl was used. PPO was extracted from freshly cut potato and banana brought from the local market. Catechol or Pyrocatechol was supplied by CDH (India), N,N dimethylene-bis-acrylamide (BIS) was supplied from Sigma. Acrylamide was supplied by SISCO Research Laboratories Pvt. Ltd (India). All other chemicals used were analytical grade and obtained from Sigma.

2.2 Instrumentation

All the test procedures were performed by an Autolab Electrochemical Analyzer (μAutolab-Type II) with General Purpose Electrochemical System (GPES) software (Ecochemie, Utrechet, The Netherlands). The three terminals e.g. working, reference and counter electrodes were connected to the analyzer interfaced with a computer.

2.2.1 Sensor fabrication

Three-electrode devices were manufactured by a multi-stage screen-printing process using a DEK 248 machine (DEK, Weymouth, UK). Devices were printed on to 250 μm thick polyester sheet (Cadilac Plastic, Sweeden, UK). The circular electrocatalytic working electrode (WE) (planar area: 0.16 cm^2) was fabricated from MCA4A, a commercially available carbon powder containing 5% rhodium (MCA Services Ltd. Cambs. UK) made into a screen printable paste by mixing 1:3 in 2.5% w/v HEC in buffer electrolyte. The sensor consisted of a reference electrode made of 15% silver chloride in silver paste (MCA) and a counter electrode made of carbon [17].

2.3 Methodology

Bulk co-polymerization of acrylamide and BIS was performed to immobilize the enzyme directly on the working electrode. The polymerization was carried out in presence of an initiator potassium peroxodisulphate ($K_2S_2O_8$).

2.3.1 Preparation of enzyme extract from potato/banana

3.5 gm of potato/ banana was weighed, cut into thin slices and a paste was prepared in a mortar pestle using 100 μl phosphate buffer (pH 6.8). This paste was centrifuged at 10,000 rpm for 2.5 mins. The clear supernatant collected was used as the enzyme solution.

2.3.2 Immobilization of enzyme on screen printed electrode

The crude extract prepared by the above method was used as the source of PPO. 20 μl of this extract was added over the working electrode (WE) of each screen-printed rhodinized graphite electrode and left at 4 ^oC for 1.5 hours. In an eppendorf tube acrylamide monomer and BIS were mixed in proper ratio and added over WE (10 μl per electrode). Then saturated initiator solution (potassium peroxodisulphate) was added (10 μl per electrode). The polymerization was carried out in an ice bath because of the high exothermic nature of the polymerization reaction. The screen printed electrode immobilized by the above method was referred as polymer electrode (PE) in this paper.

2.3.3 Preparation of catechol solution

100 ppm catechol solution was prepared by adding 1.3 mg catechol in 13 ml double distilled water and subsequent concentrations of catechol solutions were made by serial dilution using double distilled water to get 0, 1, 2, 5 10 and 15 ppm catechol.

2.3.4 Electrochemical measurement

In a three electrode system, the crude enzyme which was extracted from potato and banana was immobilized on the working electrode (PE). 100μl double distilled water was added on the PE and the response was measured by amperometry (voltage= -0.2V). After equilibration 10 μl catechol solution was added. The response was measured for 180s after addition of the substrate and the response was calculated using the General purpose Electrochemical Software (GPES).

2.3.5 Spectrophotometric measurement

In a cuvette, 2.5 ml of double distilled water and 250 μl of freshly prepared potato extract were taken, mixed well and placed in a UV-VIS spectrophotometer. It was initially made zero at 540 nm and the readings of absorbance were recorded at 1-minute intervals. Similarly, in another cuvette, 2.5 ml catechol solution of known ppm and 250 μl potato extract were taken and absorbance was recorded as discussed before. The experiment was performed for different ppm of catechol and also for banana extract as enzyme source.

2.Results and Discussion

In presence of the enzyme catechol oxidase / PPO/ tyrosinase, catechol is oxidized to benzoquinone producing a brown pigmentation as depicted in Fig.1. The more is the amount of catechol present in the solution; more is the extent of oxidation, which can be easily measured by measuring current at constant voltage i.e. by amperometric technique.

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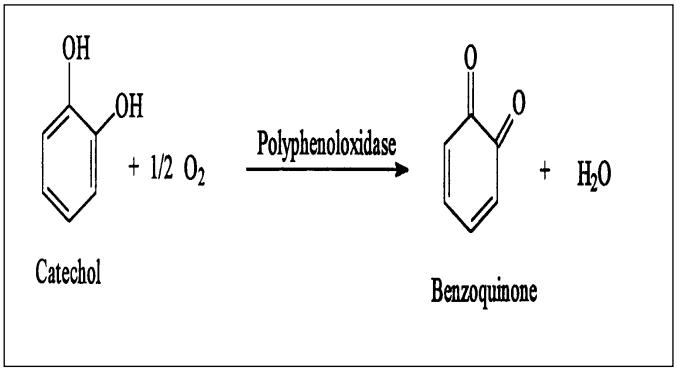

Fig. 1 Action of Polyphenoloxidase

3.1 Electrochemical measurement:

In a three electrode system, the crude enzyme which was extracted from potato and banana was immobilized on the working electrode (PE). 100µl buffer (phosphate buffer saline, pH 7) was added on the PE and the response was measured by amperometry (voltage= -0.2V). After equilibration 10 µl catechol solution was added. The response was measured for 180s after addition of the substrate and the response was calculated using the General purpose Electrochemical Software (GPES).

Fig.2 and Fig.3 are showing the amperometric response in µA with varying substrate (catechol) concentration in ppm for enzyme extracted from potato and banana respectively. Both figures are depicting the linear change of response with varying substrate concentration in 0-10 ppm range. The response increased linearly with increase in concentration of the phenol.

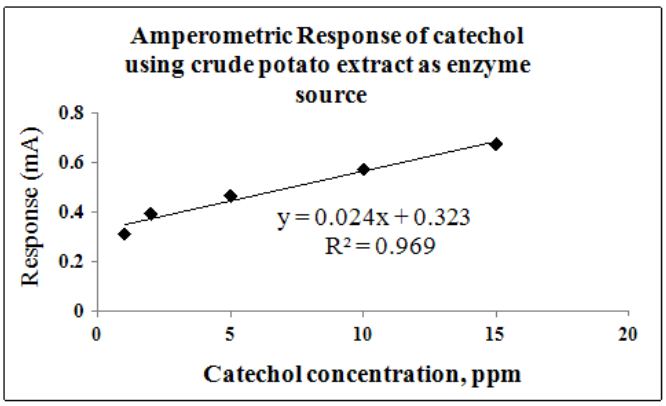

Fig. 2 Amperometric response of catechol in 0-15 ppm range using crude potato extract as the enzyme source.

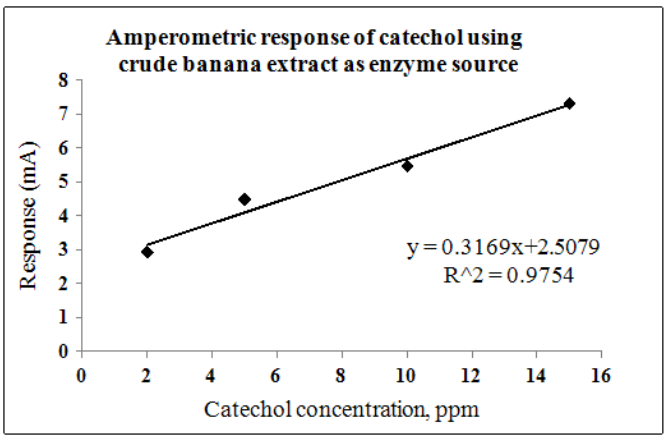

Fig. 3 Amperometric response of catechol in 0-15 ppm range using banana extract as the enzyme source.

In Fig.3, the amperometric response is higher compared to that in Fig.2 depicting the increased response in case of banana extract. This indicated that available PPO might be more in banana compared to potato.

3.2 Spectrophotometric measurement:

In a cuvette, 2.5 ml of double distilled water and 250 µl of freshly prepared potato extract were taken, mixed well and placed in a UV-VIS spectrophotometer. It was initially made zero at 540 nm and the readings of absorbance were recorded at 1-minute intervals. Similarly, in another cuvette, 2.5 ml catechol solution of known ppm and 250 µl potato extract were taken and absorbance was recorded as discussed before. The experiment was performed for different ppm of catechol and also for banana extract as enzyme source.

Figure 4 and 5 show the results of spectrophotometric study of oxidation of catechol using banana and potato extract respectively. In both cases, the absorbance value increased with increasing catechol concentration and the graphs are showing the nature of enzyme substrate reaction.

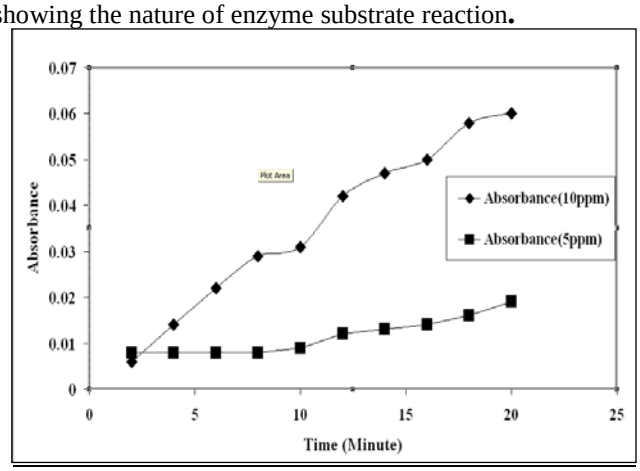

Fig. 4 Spectrophotometric analysis of the oxidation of catechol (5 & 10 ppm) using banana extract as enzyme source

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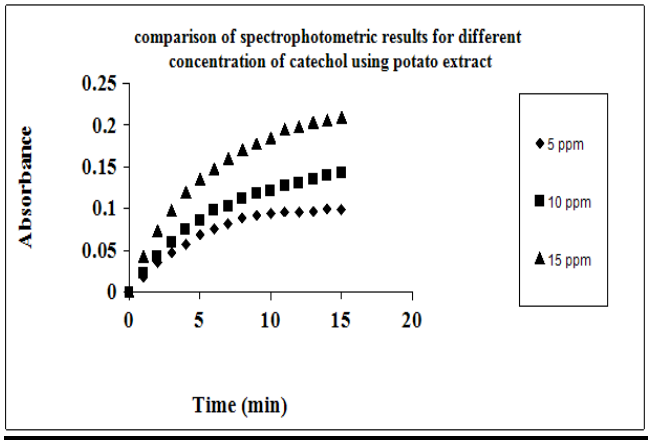

Fig. 5 Spectrophotometric analysis of the oxidation of different ppm catechol using crude potato extract as enzyme source

The results were less sensitive compared to the electrochemical study as we could not measure catechol concentration below 5 ppm in case of spectrophotometric measurement.

3.Conclusion

An effective, cheap and sensitive biosensor has been developed for real time monitoring of catechol. The enzyme polyphenoloxidase was readily extracted from potato and banana. The technique presented here gave consistent results with good correlation with the samples of known concentration. The results were also compared with the spectrophotometric analysis and found to be superior in sensitivity. The sensor can be successfully used in future for the direct determination of catechol in real samples e.g. waste water, drinking water etc.

4.Acknowledgement

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