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UGURESSA (*Flacourtia ramontchii*) TISSUE MODIFIED AMPEROMETRIC BIOSENSOR FOR DETERMINATION OF CATECHIN.

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ABSTRACT

Polyphenol oxidases from Uguressa tissues have a high catalytic activity for the aerobic oxidation of either catechin to respective *ortho*quinones. An amperometric biosensor can therefore be constructed by incorporating fruit tissue of Uguressa in a carbon paste matrix. The enzymatically-generated *ortho*-quinone was amperometrically reduced at a potential of –0.2 V (vs saturated calomel reference electrode) and hence the reduction current is directly proportional to the concentration of catechin present in the solution. The proposed biosensor provides a sensitive response (2.40×10^6 nA dm^3 mol^{-1}) for catechin in the wide linear dynamic range ($3.80 \times 10^{-5} - 3.59 \times 10^{-4}$ mol dm^{-3}), with a very fast response time (less than 2 s) and a useful lifetime of more than 25 days. Apparent Michaelis-Menten constant, K_m' was also estimated to be 2.23×10^{-4} mol dm^{-3} by using Lineweaver-Burk plot.

1.INTRODUCTION

The recognition abilities of biological organisms for foreign substances are unparalleled. Scientists have recently developed new chemical analysis tools, known as biosensors, using biochemical molecular recognition from biological organisms or receptors that have been patterned from biological systems. These devices have many favorable analytical characteristics, such as selectivity, sensitivity, portability, speed, low cost and potential for miniaturisation[1-4]. Thus, biosensors offer exciting opportunities for numerous decentralized analytical applications and they are quickly becoming useful tools in medicine, food quality control, environmental monitoring and other practical fields[2-4]. In principle, biosensors can be tailored to match individual analytical demands for almost any target molecule or compound that interacts selectively with a biological system[5].

A biosensor is usually defined as a sensing device consisting of a biological recognition element in intimate contact with a suitable transducer, which is able to convert the biological recognition reaction or the biocatalytic process into a measurable electrical signal. Enzymes are the biological components most commonly used in biosensors, while electrochemical transduction is the most popular method, often employing potentiometric or amperometric

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techniques. In potentiometric devices the analytical information is obtained by converting the biorecognition process into a potential signal, whereas the amperometric types are based on monitoring the current associated with oxidation or reduction of an electroactive species involved in the recognition process[6].

Catechins are phytochemicals composed of several linked ring-like structures. Attached to each structure are chemical tags such as phenol groups, and because there are many phenol groups, these catechins can be considered as polyphenols. Catechin is used as a potent antioxidant, in the areas of food production, agriculture, medicine, cosmetics and in research studies. [7]. The multiple phenolic groups available capture pro-oxidants and free radicals, extending the "life span" cells. Catechin has been shown not only to protect against undesirable pro-oxidant attack, but also to detoxify radicals produced from the environmental toxins. It also ranks as some of the most promising natural products for the prevention of chronic degenerative diseases, especially in the area of cancer[8].

Uguressa (*Flacourtia ramontchii*) tissues contain polyphenol oxidase (PPO) which is a copper-containing enzyme that is widely distributed in plants and other organisms. This enzyme catalyses two reactions in the presence of molecular oxygen: hydroxylation of monophenols to *ortho*-diphenols and oxidation of *ortho*-diphenols to *ortho*-quinones. In this reaction, the polyphenols have an oxygen atom added on to them in the presence of the enzyme polyphenol oxidase[9]. The oxygen itself is derived from air. It was observed in this project that polyphenol oxidation of Uguressa tissue has high activity toward catechin. As a result of enzymatic activity, catechin is oxidised to catechin-orthoquinone (Figure.1), which is

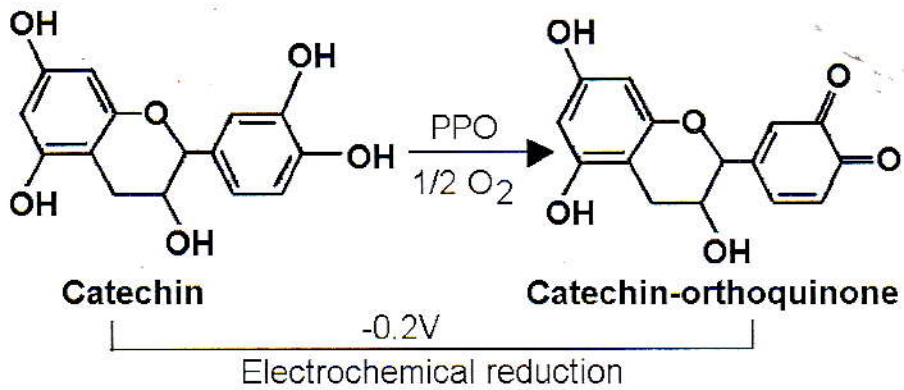

Fig 1. The biocatalytic reaction at the electrode surface.

amperometrically at – 0.020 V (vs. SCE). The resulting reduction current is directly proportional to the concentration of catechin present in the solution[10].

The main objective of this research is the construction of an amperometric biosensor using Uguressa tissue modified carbon paste electrode to detect catechin. Optimum operational conditions for this sensor, such as, tissue composition, pH, temperature and electrode potential were evaluated. Also, analytical characteristics of the sensor such as coefficient of variation, detection limit, signal to noise ratio, linear dynamic range, as well as enzyme kinetics were also investigated[11,12].

2. MATERIALS AND METHODS

2.1 *Apparatus*

Steady-state amperometric measurements were made with a CV-1b cyclic Voltammograph. Uguressa tissue modified, carbon paste working electrode, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode were used as the three-electrode system. The amperometric response was recorded on a BAS X-Y chart recorder. Amperometric detection of electrochemically-generated *ortho*-quinone was accomplished by applying a constant potential of -0.20 V (vs. SCE) and allowing the background current to decay to a steady-state value.

2.2 Reagents

Racemic mixture of catechin (±) was obtained from Sigma chemical company. Uguressa fruit was obtained from a local market. All the solutions were prepared with deionised water. Stock solution of 0.01 mol dm^{-3} catechin and Uguressa fruit tissue modified carbon paste were stored at 4 ^{0}C until use.

2.3 Procedure

0.8 g (8%) of Uguressa tissue was ground and mixed thoroughly with 4.2 g (42%) of mineral oil and 5 g (50%) of graphite powder. This carbon paste was then packed at the end of an electrode body of diameter 2.5 mm. A copper wire provided the electrical connection.
A Stock solution (0.001 mol dm^{-3}) of catechin was prepared daily and protected from sunlight. Phosphate buffer (0.1M) was used for pH optimisation. The optimum pH of this sensor was found to be 7.0; consequently all the experiments were done in this buffer.

Potential optimisation was carried out over the potential range from –0.15 V to –0.30 V vs. SCE and optimum potential was found to be -0.20 V. Therefore, all the experiments were carried out at this potential. Also, according to the optimisation of temperature, ranging from 15 ^{0}C–40 ^{0}C, 35 ^{0}C was found to be the optimum temperature. However, all the experiments were carried out at room temperature.

Calibration of the sensor was carried out with respect to catechin over the concentration range from 3.8×10^{-5} mol dm^{-3} to 3.59×10^{-4} mol dm^{-3}. The response time of the sensor was calculated from the response-time curve at the concentration of 3.8×10^{-5} mol dm^{-3} of catechin with the tissue modified electrode with a chart speed of 25 cm s^{-1}. The time required to reach 90% of maximum response (t-90) was calculated. The stability of the sensor was checked over a period of one month.

3. RESULTS AND DISCUSSION

The Uguressa tissue based carbon paste modified sensors produced a stable base-line response after allowing the time for the background current to decay. Results of the steady state amperometric response of the sensor with successive injections of catechin (in 100 µm steps) are given in Figure 6. Further, the response of blank carbon paste electrodes (without tissue) recorded over the same range of catechin concentrations was found to be insignificant compared with those of the tissue modified electrodes.

According to the optimisation process, the optimum working potential with respect to applied potential of the tissue electrode was found to be – 0.20 V vs SCE (Figure 2).

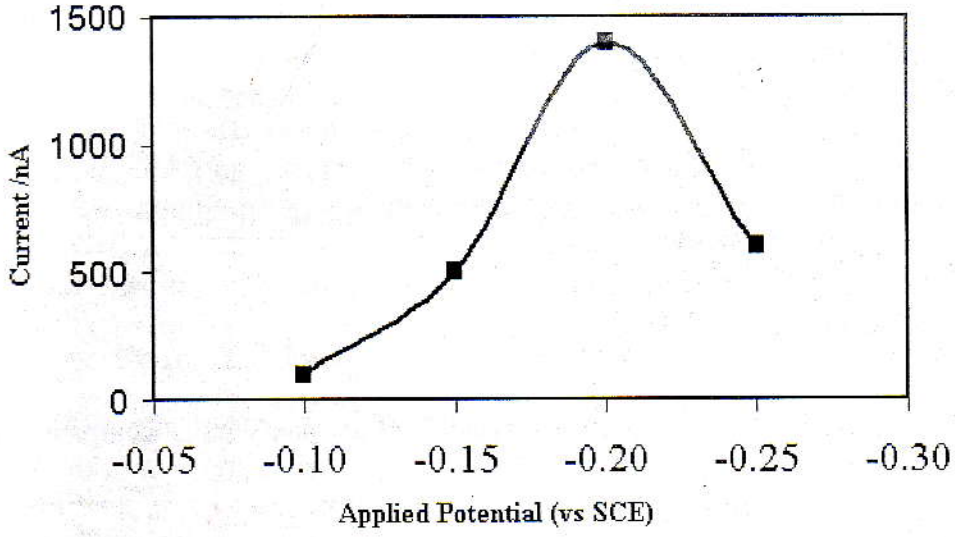

Fig..2 Sensor responses at different operating potentials.

The optimum tissue composition was found to be 8 % (Figure 3). Optimum pH of the sensor was found to be 7 (Figure 4) and optimum-working temperature was found to be 35 ^{0} C (Figure 5).

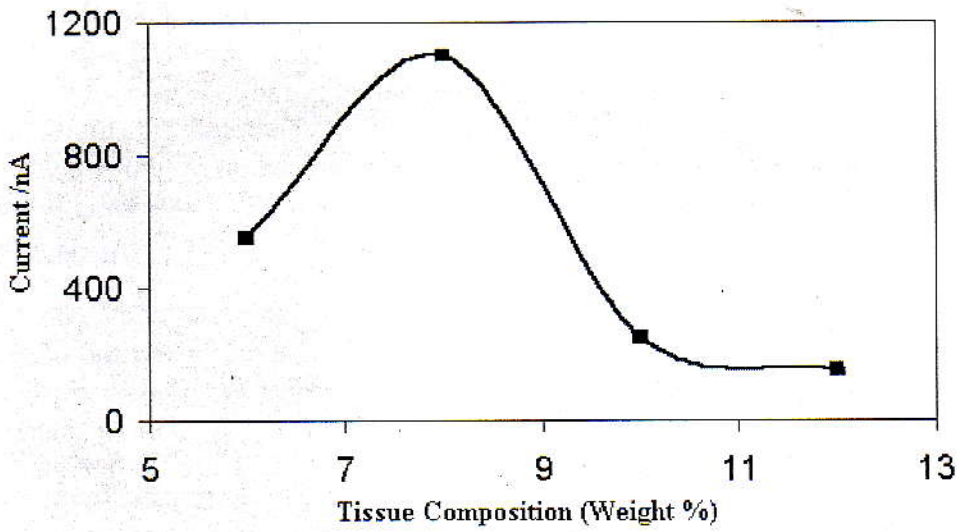

Fig. 3 Sensor responses at different tissue compositions.

The calibration graph obtained for catechin with the Uguressa fruit tissue based sensor showed linear response over the concentration range $3.80 \times 10^{-5} - 3.59 \times 10^{-4}$ mol dm^{-3} as shown in the inset of Figure 6 together with corresponding amperometric responses.

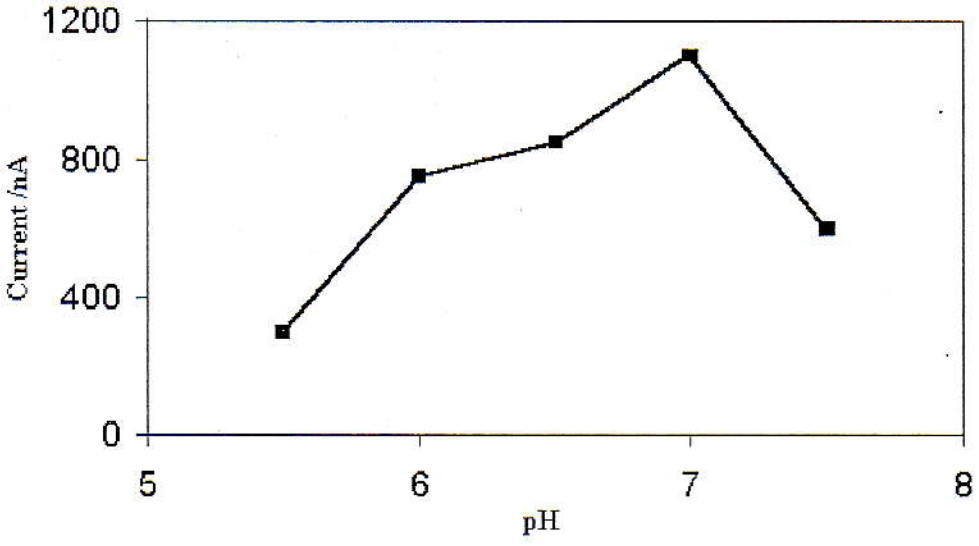

Figure. 4 Sensor responses at different operating pH values.

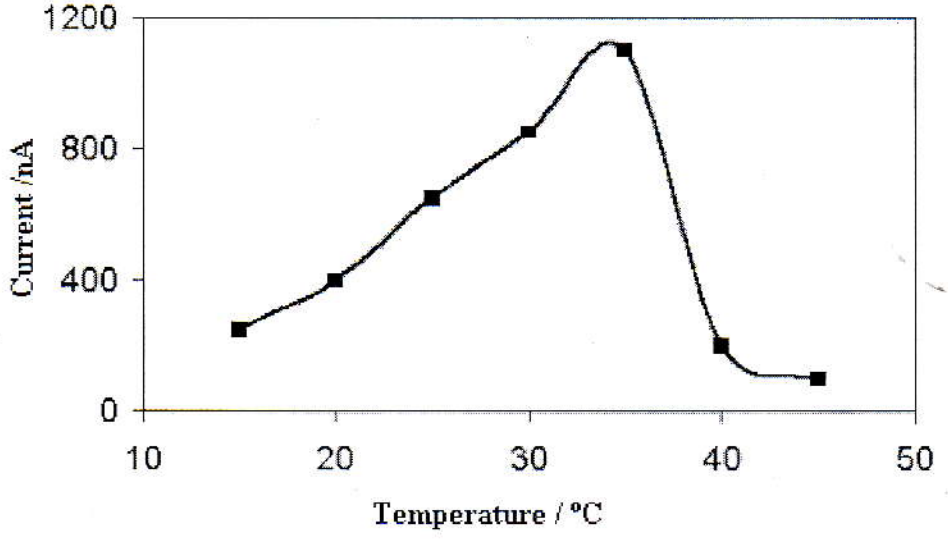

Fig. 5 Sensor responses at different operating temperatures.

The response sensitivity of the sensor (slope of the calibration graph) is estimated to be 2.40 $\times 10^6$ nA dm^3 mol^{-1}. The relative standard deviation for the sensor was 4.33% for twenty measurements carried out with a 3.8×10^{-4} mol dm^{-3} catechin solution. The steady state response time of the sensor (t_{-90}) was estimated to be 1.8 s.

V_{max} (at 8% tissue) and the apparent Michaelis-Menten constant K_m, of the polyphenol oxidase with catechin, as estimated by the Lineweaver-Burk plot (double reciprocal plot) given in Figure 7 are 1.52×10^3 nA s^{-1} and 2.23×10^{-4} mol dm^{-3} respectively. In this study, a racemic mixture of catechin (±) and (+) catechin was used to obtain the amperometric response.

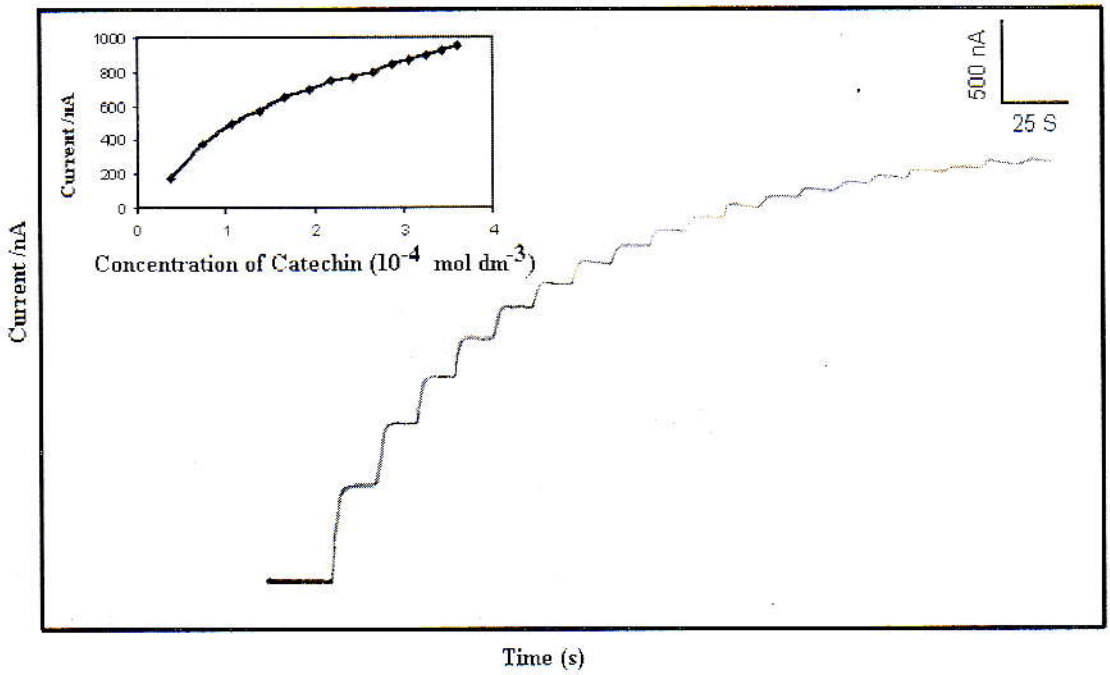

Fig. 6 Amperogram of the Uguressa tissue based sensor with increasing concentration of catechin in 4×10^{-4} mol dm^{-3} steps. Also shown (inset) is the resulting calibration graph.

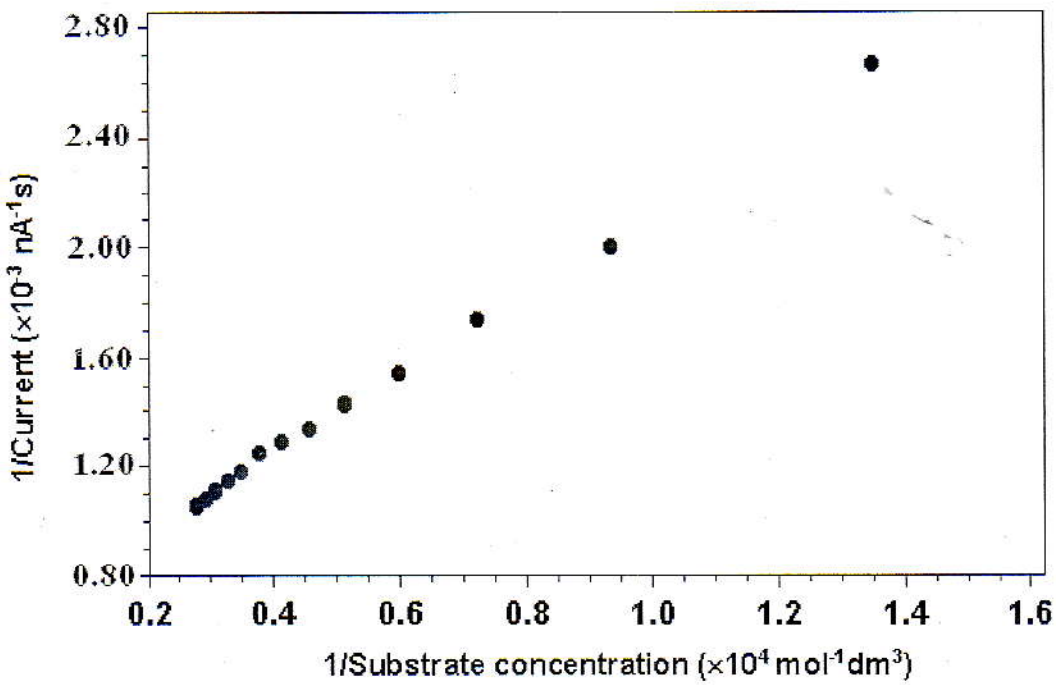

Fig.7 Lineaweaver-Burk plot obtained with Uguressa tissue modified electrode for Catechin.

However, amperometric experiments conducted with (+)-catechin alone indicated that the sensor produced more or less similar amperometric responses, for both racemic catechin (±) and (+) catechin indicating that the sensor is not specific to one isomer.

The long-term stability of the Uguressa tissue modified sensor is illustrated in Figure 8. This shows that the sensor exhibits remarkable long-term stability of more than one month.

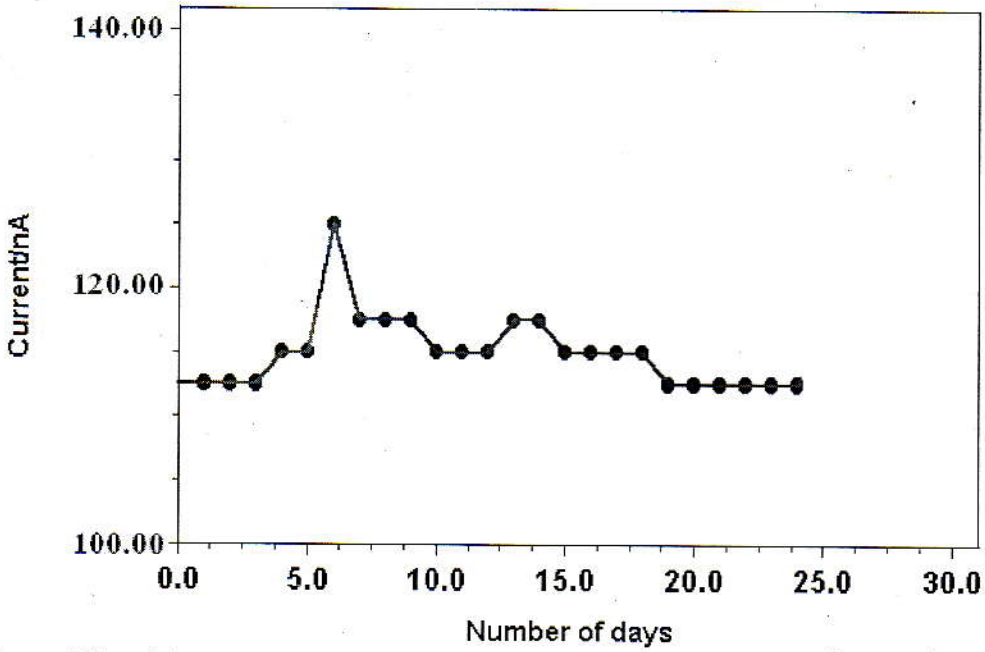

Fig.8 Time stability of the Uguressa tissue modified sensor with response to 3.8×10^{-5} mol dm^{-3} of Catechin.

3. CONCLUSION

Uguressa tissue-modified amperometric biosensor has been constructed and characterised for the detection of catechin with desirable analytical characteristics such as sensitivity, response time, relative standard deviation, signal/noise ratio, detection limit and lifetime stability. The V_{max} and the apparent Michaelis-Menten constant $K_{m'}$ of the Polyphenol oxidase with catechin are 1.52×10^{3} nA s^{-1} and 2.23×10^{-4} mol dm^{-3} respectively. Also the amperometric experiments conducted with (±) catecin and (+) catechin indicated that the sensor produced more or less similar amperometric responses for both (±) catechin and (+) catechin indicating that the sensor is not specific to one isomer.

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