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PREPARATION OF POLY(PYROMELLITIC DIANHYDRIDE-CO-THIONIN) MODIFIED VOLTAMMETRIC SENSOR FOR THE DETERMINATION OF EPICATECHIN

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Abstract: In this study, the electrochemical oxidation of epicatechin and its voltammetric sensing is shown at a sensitive platinum electrode modified with poly(pyromellitic dianhydride-co-thionin). The electrochemical response of the sensor was improved in the presence of both electro-inactive (sucrose, fructose, lactose) and electroactive (gallic acid, cafeic acid, ascorbic acid) interferants and displayed an excellent analytical performance for the determination of epicatechin. A linear response was obtained over a range of epicatechin concentrations from 0.05 mM to 0.30 mM and was shown to be useful for quantifying low levels of epicatechin in phosphate buffer solution, PBS, pH 7.00. Regression coefficient (R2) was found to be to be 0.9969. Limit of detection (LOD) was calculated as 1.8×10^{-5} M by using 3s/m. Where m is the slope of the calibration curve and s is the standard deviation of the calibration graph calculated using the Excel Steyx function.

Keywords: Modified electrode, Polyimide, Sensors, Epicatechin, Voltammetry.

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INTRODUCTION

Electrochemical sensors, providing a crucial analytical tool for rapid, sensitive and selective determination of various kinds of analytes, have been widely applied in many fields, such as detection of water quality, clinical chemistry and food quality control. Researchers are mainly working on the design and preparation of various polymeric materials to modify electrodes and the modified electrodes then be used for better electrochemical sensing (1). Polymer modified electrodes have received a great attention in recent years (2). Electrochemical detection of an analyte is a very elegant method in analytical chemistry. An interest in developing electrochemical sensing devices to be used in environmental monitoring, clinical assays or process control is growing rapidly.

Catechins are compounds regarded as important for human health. Epicatechin (EC) is a polyphenol with strong antioxidant properties found in vegetable sources, especially in green tea, cocoa, and grapes. Epicatechin and its derivatives have been shown to possess antibacterial, antiviral, anti-allergic, anti-inflammatory, anti-aging and anti-tumor properties, as well as inhibiting tumor growth, preventing wrinkling, eliminating heavy metal toxicity, reducing bacterial inflammation, improving immunity and exhibiting anti-rotavirus activity (3, 4). Additionally, epicatechin pretreatment has been reported to prevent mice exposed to gamma rays from causing more damage to the liver (5) and testis (6). In other a study the radio protective property of such phytochemicals has been evaluated, epicatechin, belonging to the group of flavanols, is one of the most potent antioxidants present in the human diet predominantly in grapes, tea, apples and cocoa (7). Studies conducted by some researchers indicate that epicatechin may have a great influence on factors related to cancer metastasis (8).

It is very important to develop a simple, sensitive, accurate and sensitive method for the determination of epicatechins from food, medicine and cosmetic products. For this purpose, many methods such as nuclear magnetic resonance spectroscopy (NMR) (9), high performance liquid chromatography (HPLC) (10-13) thin layer chromatography (TLC) (14-17). Spectrophotometry (18, 19), GC-MS (20) and electrophoresis (4, 21) are used. However, these methods have some disadvantages such as the difficult sample preparation and measuring steps, time consuming and costly. However, in the determination of epicatechin, electrochemical methods have important advantages such as rapid response time, low detection limit, low cost, high reproducibility (22) and film thickness controllability (23). In recent years, polymer coating on electrodes namely electrode modification methods has shown considerable potential for application in the field of sensors (24). The electrochemical detection of epicatechin through adsorptive stripping voltammetry on poly(3,4-ethylenedioxythiophene)-modified Pt electrodes was investigated (25). The mechanism of electrochemical oxidation of epicatechin (EC) on a glassy carbon electrode was investigated over a wide range of conditions, using cyclic and square-wave voltammetry (26).

The aim of the present study was to develop an electrochemical method that will have important advantages such as rapid response time, low detection limit, low cost and high reproducibility for the determination of epicatechin. For this aim, polyimide membrane based voltammetric sensors were developed for epicatechin determination. As the polyimide membrane, poly(pyromellitic dianhydride-co-thionin) (PI), which has a very high film forming property was used to remove the interferants from the electrode surface in the analysis medium. In recent years, polyimide-based materials have attracted great interest, both in industry and in academia, because they exhibit unique properties not shared by conventional polymers (27). Polyimides have important advantages such as physical properties, adhesive, thermal stability and mechanic strength (28, 29). For this reason, polyimides are widely used in the field of sensors as permselective membranes (27, 28).

EXPERIMENTAL

Reagents and Apparatus

Reagents were of analytical grade or of the highest commercially available purity. The polymer, poly(pyromellitic dianhydride-co-thionin), used for the modification was purchased from Sigma-Aldrich. Epicatechin solutions in 0.1 M phosphate buffer solution (PBS) at pH=7.00 were prepared just before running each experiment. Gallic acid, cumaric acid, ascorbic acid, and epicatechin from Sigma-Aldrich were used. Ultra-pure water was obtained from the Millipore brand Elix 20 model water system.

BAS (Bioanalytical System Inc.) 100BW electrochemical analyzer with C2 cell stand was used for voltammetric measurements. pH measurements were performed by using a HI 2211 model pH/ORP Meter. pH meter was frequently calibrated using standard pH buffers obtained from Merck. A KERN model ABJ-NM/ABS-N electronic balance was used for weighing. In a classical three electrode cell configuration working electrode was Platinum electrode (MF 2014). A platinum wire electrode (BASI MW-1032) was used as auxiliary together with an Ag/AgCl (3 M KCl) reference electrode (CHI111). Platinum electrode was mechanically cleaned using 15, 3 and 1 µm aqueous diamond pastes successively on diamond polishing pads and 0.05 µm alumina slurry on alumina polishing pads. After each polishing operation electrodes were rinsed with distilled water and ultra-sonicated 2-3 minutes in an ultrasonic bath (Branson model 3510).

All glassware including electrolysis cells were kept in 6 M HNO_3 overnight to remove impurities.

RESULT AND DISCUSSION

Preparation of poly(pyromellitic dianhydride-co-thionin) Electrodes

Before starting chemical modification of electrodes, the surface of working electrode was carefully cleaned and polished as described above. Firstly, about 0.1 g of poly(pyromellitic dianhydride-co-thionin) is dissolved in 1 mL of n-methylpyrrolidone (NMP). Then, different volumes (1, 2, 3, 4 and 5 µL) of the poly(pyromellitic dianhydride-co-thionin) solution were dropped on the surface of bare platinum working electrodes. Afterwards poly(pyromellitic dianhydride-co-thionin) film was dried at room temperature for at least 72 h.

The oxidation steps of EC were located on benzene ring containing two hydroxyl groups on 3' and 4'-position, which represents a typical redox system with two electron oxidation process as showed in Figure 1.

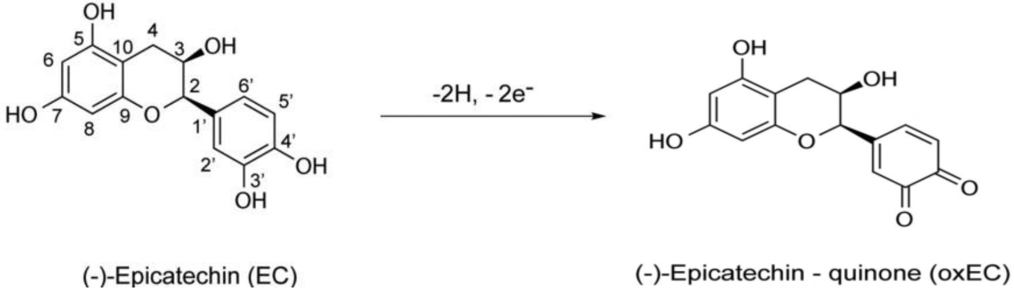
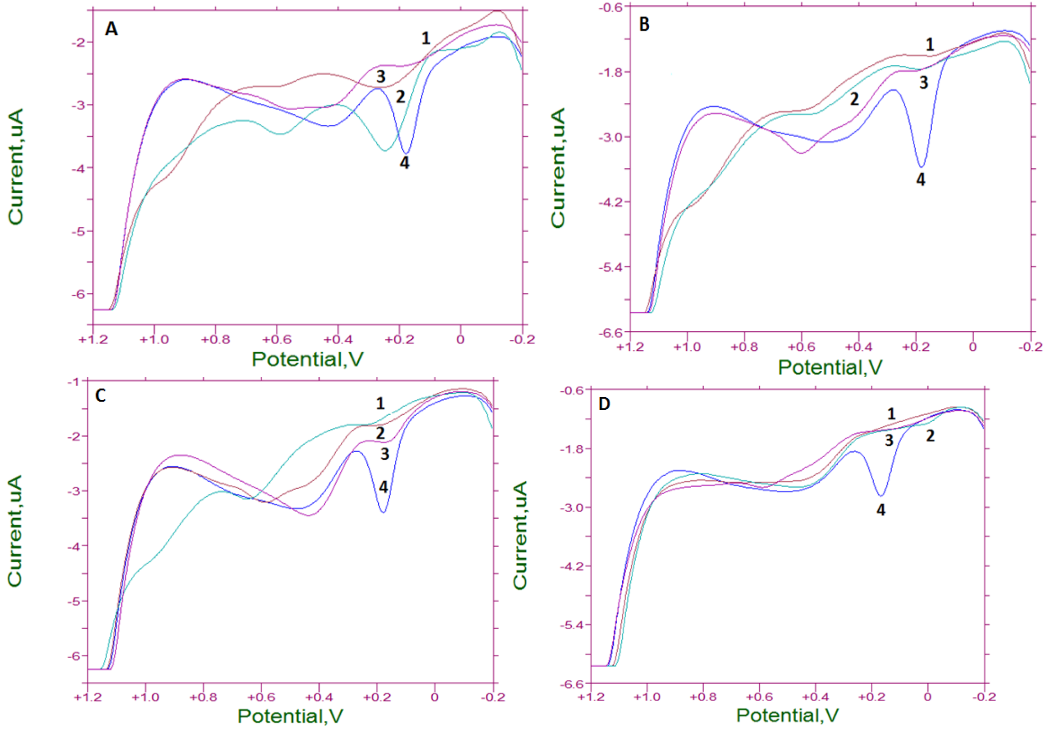


Figure 1. Oxidation mechanism of EC.

Effect of Polyimide (PI) Film Thickness

The most effective parameter that determines the permselectivity character of polymeric films is the thickness. At first, platinum electrodes were coated with by dropping different volumes (1, 2, 3, 4, and 5 µL) of poly(pyromellitic dianhydride-co-thionin) onto Pt electrode surfaces. Afterwards, DPV responses of the electrodes towards ascorbic acid (AA), gallic acid (GA), caffeic acid (CA) and epicatechin (EC) injections were separately measured for each film thickness in 0.1 M PBS solution (pH=7.0). Injected volumes of each analyte were carefully calculated so that final concentrations of the analytes were 2 mM. All of the solutions, regardless of what kind of analyte it contained and what the polymer film thickness was, also contained a mixture of lactose, sucrose and fructose, each at a concentration of 2 mM. Figure 2 shows the effect of PI film thickness upon DPV peak magnitudes of epicatechin and the electroactive interferants (AA, GA and CA) on electrodes coated at 5 different thicknesses. It is seen that at relatively thin film coatings electroactive interferants produced responses, although much smaller than that of EC, almost at the same potential as with EC. However, their responses at that potential diminished slowly with the increasing film thickness. At a film thickness of 4 µL PI it was evident that electroactive species did not interfere with the EC signal. It must be stated here that the same species are known to exhibit electroactivity with a bare Pt electrode. Furthermore, no interference effect was detected from nonelectroactive species of lactose, sucrose and fructose. As a result, optimal coating for electrochemical detection of epicatechin was that performed with 4 µL of PI.



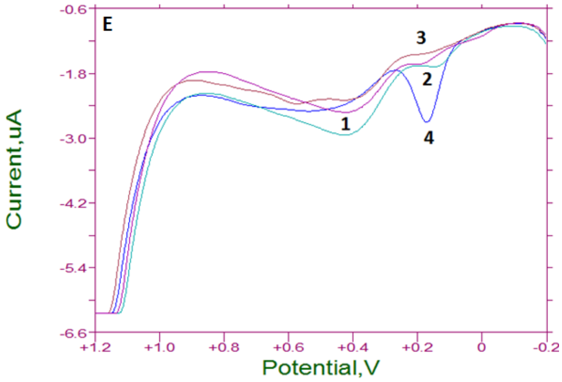


Figure 2. Effect of the **A:**1, **B:**2, **C:**3, **D:**4, **E:**5 µL PI film thickness on the DPV of 2 mM **1:** AA, **2:** GA, **3:** CA, **4:** EC in PBS pH=7.00 containing 2 mM lactose, sucrose and fructose.

Effect of Scan Rate

To see the effect of scanning rate on epicatechin signal, DPVs of 2 mM epicatechin at different scan rates were taken on PI electrode of optimal thickness in PBS pH 7.00.

The peak currents of epicatechins obtained under these conditions are shown in Table 1. As shown in Figure 3 that optimum scan rate is 20 mV/s. It is clear that the peak currents are smaller at higher and lower scan rates.

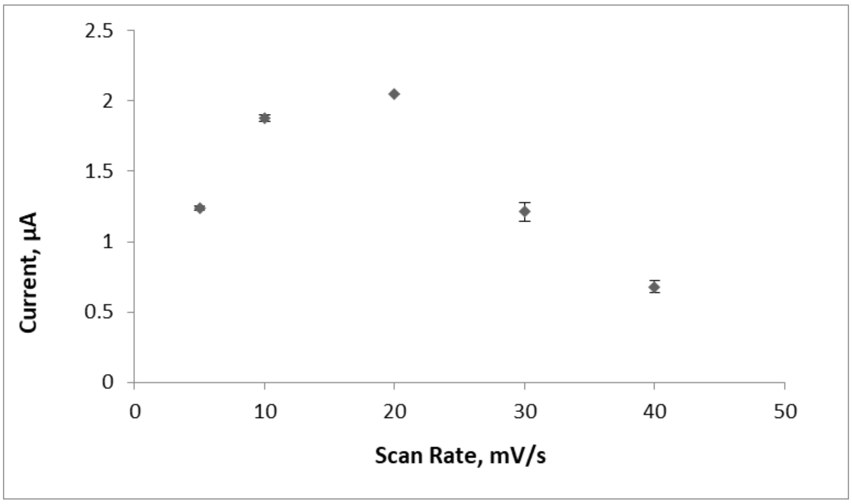


Figure 3. Effect of scan rate on epicatechin responses.

Repeatability and Stability

To test the repeatability of EC response and special stability of EC peaks on voltammograms obtained with the PI-based electrodes, fifteen scans were successively run using the same PI electrode in 0.1 M PBS (pH = 7.00). Results are shown for both high concentration (4 mM) and low concentration (0.02 mM) of epicatechin in Figure 4A and B, respectively. It can be deduced that repeatability of signal magnitude was quite high and stability with regard to EC peak shape and drift in voltammograms were extremely satisfactory.

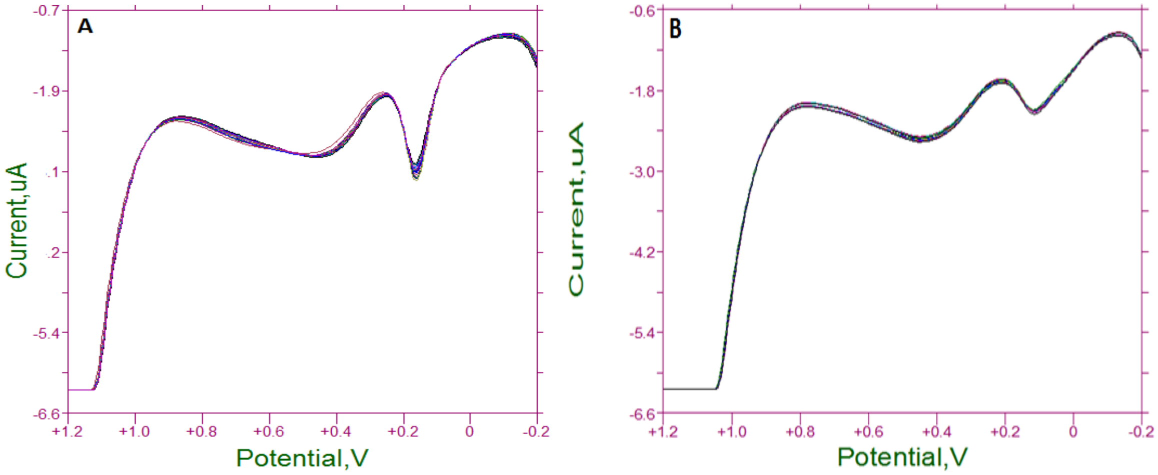


Figure 4. Stability on the same PI modified electrode in 0.1 M PBS with pH=7 at **A:** 4 mM, **B:** 0.02 mM epicatechin concentration (N=15).

Calibration curve for PI Electrodes

Calibration studies were performed employing PI modified electrodes coated with optimum PI film thicknesses. DPV signals for increasing epicatechin concentration measured in 0.1 M PBS (pH=7) are shown in Figure 5. As expected, epicatechin signal was shown to grow with increasing concentration.

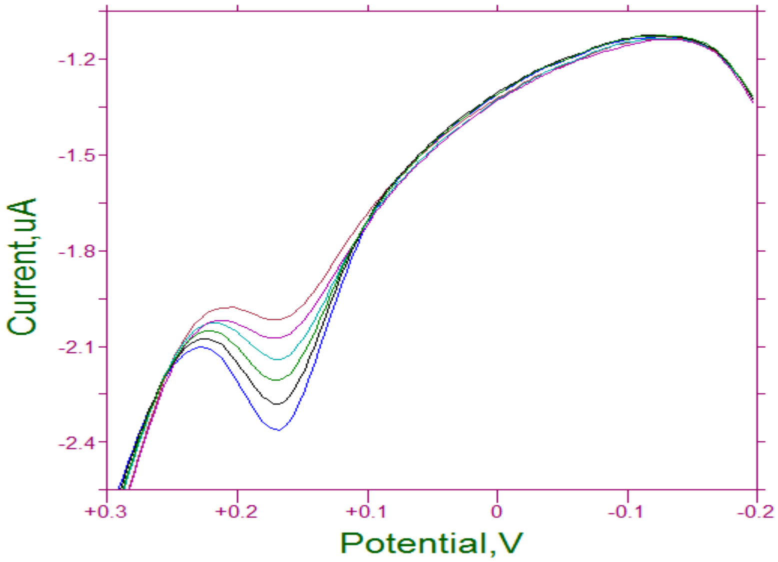


Figure 5: DPV of 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mM epicatechin in PBS pH=7.00.

The measured peak currents are shown in Table 1 and the calibration graphs are shown in Figure 6 with R^2 0.9969.

Table 1. Calibration data.

C, mM	0.05	0.10	0.15	0.20	0.25	0.30
I, μA	2.010±0.003	2.066±0.026	2.150±0.006	2.221±0.007	2.277±0.022	2.362±0.021

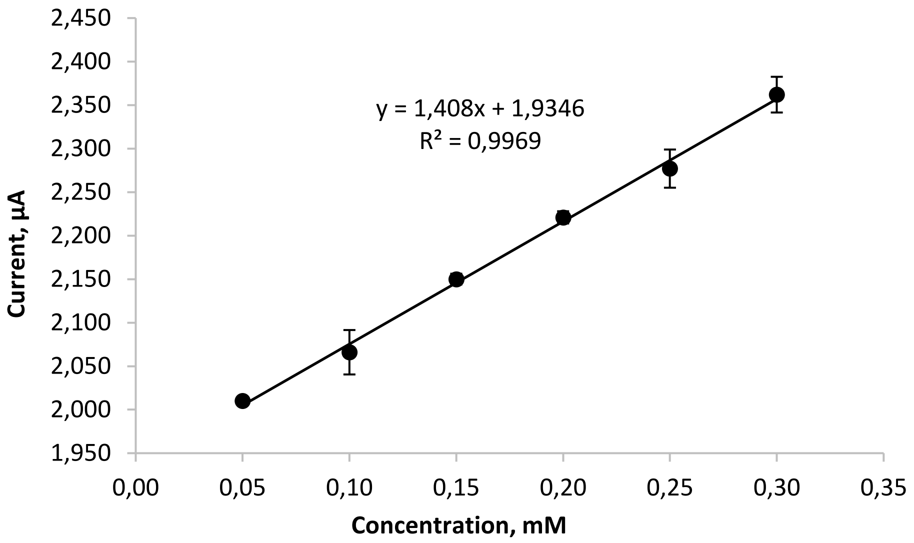


Figure 6. Calibration graph of epicatechin in PBS pH=7.00.

As the Fig 6 shows a linear response was obtained over a range of epicatechin concentrations from 0.05 mM to 0.30 mM in PBS with the indication that this electrode and method described in this work is a useful one for quantifying low levels of epicatechin in phosphate buffer solution, PBS, pH 7.00. Regression coefficient (R^2) was found to be 0.9969. Limit of detection (LOD) was calculated as 1.8×10^{-5} M.

The comparison of the properties of this sensor that was developed in this work with those mentioned in literature is shown in Table 2.

When Table 2 is examined, it can be stated that analytical parameters of the developed sensor, which involves no adsorption, deposition or incubation of the analyte on the electrode, is compatible with the data reported in literature.

CONCLUSION

A voltammetric electrode modified with polyimide membrane was prepared for the detection of epicatechin. In brief, our results demonstrate that poly(pyromellitic dianhydride-co-thionin) film can be easily coated on Pt electrode surfaces and that electrode with this polymeric film is extremely efficient in preventing the permeation of the mentioned electroactive or nonelectroactive interfering substances while allowing epicatechin passage through the film.

We believe that this polymeric film can be used as an inner / protective and a permselective membrane in epicatechin based sensor construction. This sensor has many important advantages such as rapid response time, low detection limit, low cost and high reproducibility for determination of epicatechin.

Table 2. The comparison of the properties of sensor with the literature.

Analyte	Electrode	Method	Polymer	Linear Range, M	R^2	LOD, M	pH	Reference
Epicatechin	Pt	AdSV	PEDOT	$6.9x10^{-7}$ to $8.6x10^{-6}$	0.980	-	7	(25)
Epigallocatechin gallat	MIP modified GC	DPV	MIP/GO/GC	$3x10^{-8}$ to $1x10^{-5}$	0.9989	$8.78x10^{-9}$	6	(23)
Epicatechin gallate Epicatechin	GC	SWV	-	$1x10^{-8}$ to $1x10^{-5}$	0.9997 0.9997	$3.26x10^{-7}$ $4.27x10^{-6}$	2	(26)
Epicatechin	CD	CE-ED	-	$5x10^{-7}$ to $5x10^{-5}$	0.9960	$4x10^{-7}$	7.6	(21)
Epicatechin	Pt	DPV	PI	$5x10^{-5}$ to $3x10^{-4}$	0.9969	$1.8x10^{-5}$	7	This work

(**AdSV:** Adsorptive stripping voltammetry, **CE-ED:** Capillary zone electrophoresis with electrochemical detection, **CD:** Carbon disc **GC:** Glassy carbon, MIP/GO/GC: Molecular imprinting polymer/graphene oxide/glassy carbon, **PEDOT:** poly(3,4-ethylenedioxythiophene), **PI:** poly(pyromellitic dianhydride-co-thionin))

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