Materials Science and Engineering C 73 (2017) 552–561

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Facile preparation of poly(methylene blue) modified carbon paste electrode for the detection and quantification of catechin

ManasaG [a], Ronald J Mascarenhas [a,*], Ashis K Satpati [b], Ozma J D'Souza [a], DhasonA [c]

[a] Electrochemistry Research Group, Department of Chemistry, St. Joseph's College, Lalbagh Road, Bangalore, 560027, Karnataka, India
[b] Analytical Chemistry Division, Bhabha Atomic Research Centre, Anushakthi Nagar, Trombay, Mumbai 400094, Maharashtra, India
[c] Soft Condensed Matter, Raman Research Institute, Sadashivnagar, Bangalore 560080, Karnataka, India

ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 20 December 2016
Accepted 21 December 2016
Available online 24 December 2016

Keywords:
Modified carbon paste electrode
Methylene blue
Electropolymerization
Catechin
Differential pulse voltammetry

ABSTRACT

Free radicals are formed as byproducts of metabolism, and are highly unstable due to the presence of unpaired electrons. They readily react with other important cellular components such as DNA causing them damage. Antioxidants such as (+)-catechin (CAT), neutralize free radicals in the blood stream. Hence there is a need for detection and quantification of catechin concentration in various food sources and beverages. Electro-oxidative properties of catechin were investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A carbon paste working electrode modified by electropolymerizing methylene blue (MB) was fabricated. Field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM) techniques were used to study the surface morphology of the electrode. Quasi-reversible electron transfer reaction occurred at +0.260 V through a diffusion controlled process. In comparison to the bare carbon paste electrode (CPE), there was a significant 5.3 times increment in anodic current sensitivity at the modified electrode at physiological pH. Our findings indicate that for the electro-oxidation of CAT, CPE is a better base material for electropolymerization of MB compared to glassy carbon electrode (GCE). Nyquist plot followed the theoretical shape, indicating low interfacial charge transfer resistance of 0.095 kΩ at the modified electrode. Calibration plots obtained by DPV were linear in two ranges of 1.0×10^{-3} to 1.0×10^{-6} and 1.0×10^{-7} to 0.1×10^{-8} M. The limit of detection (LOD) and limit of quantification (LOQ) was 4.9 nM and 14 nM respectively. Application of the developed electrode was demonstrated by detecting catechin in green tea and spiked fruit juice with satisfactory recoveries. The sensor was stable, sensitive, selective and reproducible.

1. Introduction

Polyphenols are naturally occurring secondary metabolites in plants, with potential antioxidant properties [1]. Dietary polyphenols have profound health benefits reaped from their reductive ability [2]. They effectively scavenge most of the oxidizing molecules, including hydroxyl anions, hydrogen peroxide, superoxides and singlet oxygen. Based on their phenolic structural features, polyphenols are classified into groups, which include flavonoids, stilbenes and phenolic acids [3]. Flavanols compose the subgroup of the most diversely found flavonoids, structurally composed of two aromatic rings (A and B) linked through three carbon atoms which form an oxygenated heterocyclic ring (C) [4]. Their exceptional antioxidant property is enhanced due to the oxidation of the catechol hydroxyl groups. The main sources of flavanols [5] are obtained from fruits (apples, apricots, pears, cherries, peaches and plums), vegetables (onions, soyabean), cocoa (dark chocolates), black and green tea, herbs, roots, red wine and beer [6]. Flavanols are further divided into monomers (catechins) and polymers (proanthocyanidins, theaflavins and thearubigins).

Free radicals commonly referred to as reactive oxygen species (ROS) and reactive nitrogen species are formed by the response of the immune system to environmental factors and also as a result of ongoing biochemical processes in the body [7]. They possess an unpaired electron making them highly unstable and reactive with the neighboring molecules or cells. These ramping free radicals trigger a chain reaction, causing havoc in the body, damaging cellular components especially the nucleic acids, lipids and proteins [8]. DNA damage can possibly cause mutations and cancer, if not reversed by DNA repair mechanisms. Meanwhile damage to proteins causes denaturation, enzyme inhibition and protein degradation. This results in the progressive dysfunction of various processes in the body. According to most researchers, free radicals are the culprits behind every disease known to this day [9]. An imbalance between free radicals and antioxidants leads to a critical condition termed as oxidative stress. Sustained oxidative damage only worsens the system contributing to diseases and faster aging process [10].

* Corresponding author.
E-mail address: ronaldmasc2311@yahoo.co.in (R.J. Mascarenhas).

http://dx.doi.org/10.1016/j.msec.2016.12.114
0928-4931/

In order to prevent the damage caused by these ROS, the body has a defense system of antioxidants. While the antioxidant system does not remove free radicals entirely, they nevertheless keep them at an optimum level [11]. Although some antioxidants such as glutathione, vitamin C and beta carotene are synthesized within our body, others have to be obtained through dietary supplements [12].

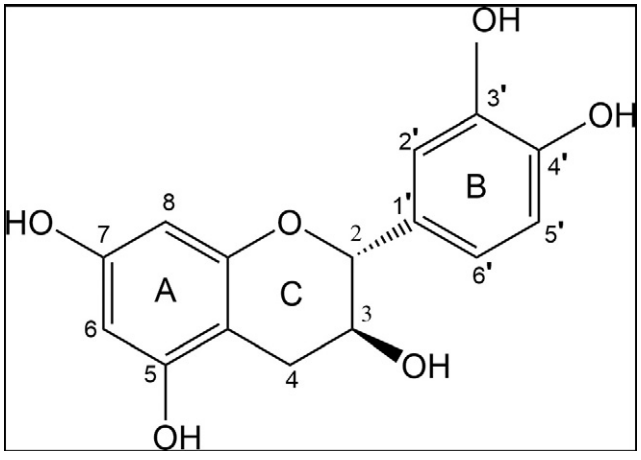
Antioxidant flavan-3-ol, commonly known as (+)-catechin (CAT) can terminate such deterioration of vital molecules in the body by quenching free radical reactions. A flavanol monomer, CAT is composed of two pharmacophores (Scheme 1). Ring A is the resorcinol moiety and ring B is the catechol moiety that is responsible for the antioxidant property of CAT [13]. Catechols, unlike the free radicals, are prone to oxidation and thus donate an electron or hydrogen and stabilize themselves.

Most of the beneficial health effects of CAT are attributed to their redox properties and free radical scavenging abilities [14]. In recent years, a large number of studies have found that at physiological pH catechin suppressed DNA strand breakage by hydroxyl radicals, thereby retarding tumor growth. CATs have profound inhibitory activities, against inflammation, neurodegenerative disorders [15], chelating redox transition metal ions [16], and suppressing platelets adhesion. They also act against lipid peroxidation and low-density lipoproteins; hence they are good at warding off type 2 diabetes mellitus [17,18] and coronary heart diseases. Reports have proven that CAT is a more potent antioxidant than ascorbate or α-tocopherol as demonstrated by vitro assays of lipid peroxidation [19].

Quantitative analysis of CAT has been carried out by several methods like fluorescence analysis, chronocoulometry, gas chromatography [20], high performance liquid chromatography [21], chemiluminescence [22], spectrophotometry [23], flow column electrolysis [24] and mass spectroscopy [25] all of which are sophisticated to handle, expensive and time-consuming techniques. A promising alternative in this regard, is the comparatively more robust method of electrochemical detection. Electrochemical method aids in the real time detection of the analyte under analysis and reveal their electron accepting/donating abilities. The scope of this method can be witnessed in the quantification of wide range of organic and inorganic biomolecules. In order to improvise the analytical response, innumerable novel modified sensors have been explored and developed [26–29]. Thus, the study favors understanding of the electrochemical behavior of the analyte under investigation. This further paves way for quantification of a particular molecule in biological samples which are usually present in trace concentrations. Hence, electrochemical studies offer numerous applications in environmental monitoring and food analysis [30].

The purpose of this work was to develop a user friendly, quick response and high accuracy sensor which could be applicable even at ambient conditions for the detection of CAT molecule, well known for its exceptional health benefits. Chemically modified carbon paste electrodes [31,32] are more advantageous as they can be easily fabricated, produce low residual current and noise, have good reproducibility, are chemically inert, provide rapid renewal of surface and are most cost effective compared to other electrodes [33–35]. In addition to these, the modified electrodes are upgraded in their physical and electrocatalytic properties. The literature survey revealed that methylene blue modified CPE had so far not been used in the determination of CAT.

Electropolymerization is a facile method to prepare surface modified carbon paste electrodes [36,37]. Methylene blue is a water soluble cationic dye and an excellent redox mediator [38] owing to the presence of electron rich sulphur and nitrogen heteroatoms in their structure. The film prepared by electropolymerization offers many advantages over other deposition techniques in terms of better thickness control, simple preparation methodology, possess higher stability and is economical [39]. The heterocyclic conjugate system structure of MB exhibits π-π noncovalent interaction with sp^2 hybridized graphite carbons. It has been suggested that because of the planar aromatic structure of MB, it strongly anchors to the graphite surface through π-π electrostatic interactions [40]. In addition, the irreversible electrooxidation of MB led to the formation of the polymeric film and the mechanism of polymerization process involved the formation of a cation radical. The film exhibited high conductivity, stability and excellent electrocatalytic activity which resulted in increase in anodic peak current and decrease in anodic potential. The surface morphology of the modified electrode was established by FESEM and AFM images. The response of CAT at GCE was electrochemically irreversible while it was quasi-reversible at CPE. Further, our findings illustrate that CPE is a better base material for electropolymerization of MB as contrasted with GCE. Finally, the merit of this sensor was evaluated by successfully applying it to real sample analysis which yielded satisfactory recoveries.



Scheme 1. Structure of (+)-Catechin.

2. Experimental

2.1. Chemicals

CAT of 98% purity was purchased from Sigma–Aldrich, India. Methylene blue (MB), ascorbic acid (AA), H_3PO_4, NaOH, KH_2PO_4 and silicone oil were from Merck, India. Tryptophan (Trp) and absolute ethanol were obtained from SRL, India. The graphite powder was supplied by Graphite India Ltd. CAT solution was prepared in absolute ethanol. Stock solutions of MB, AA and Trp were prepared using ultrapure water (~18.2 MΩ cm) from Milli–Q Plus system (Millipore). Ultrapure water was used in the preparation of aqueous solutions, rinsing of the glassware and electrodes. Phosphate buffer solutions (PBS) from pH 3.0 to pH 9.0 were prepared with 0.1 M NaOH and 0.1 M KH_2PO_4 solutions. The pH was adjusted using H_3PO_4 or NaOH.

2.2. Instruments

All electrochemical measurements as well as the electrochemical impedance study were performed using an electrochemical work station from CH instruments; model CH-660E (Inc., USA). The experimental conditions were controlled with General Purpose CH-660E software. The pH of the buffer solutions was measured using digital pH/mV meter Model ELICO Li 614 (India), equipped with a combined glass electrode, which was calibrated using standard pH 4.0 and pH 7.0 buffer solutions. Voltammetric experiments were performed using the conventional three electrode system within a single compartment cell with PMB/CPE as working electrode, a platinum wire as an auxiliary electrode and KCl-saturated calomel electrode as reference electrode. In order to minimize error due to IR drop in the electrolyte, the lugin capillary of the reference electrode was kept closer to the surface of the working electrode. The surface morphology of the electrodes were studied with FESEM and EDX using Quanta 200, FEI, Germany; Supra40vp, Gemini, Zeiss, Germany and AFM images were obtained from Molecular