Materials Science and Engineering C 37 (2014) 321–326

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Molecularly imprinted polymer based electrochemical detection of L-cysteine at carbon paste electrode

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ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form 21 December 2013
Accepted 8 January 2014
Available online 15 January 2014

Keywords:
Molecular imprinted polymer
L-cysteine
Carbon paste electrode
Ferro-ferricyanide

ABSTRACT

A methacrylic acid (MAA) based molecularly imprinted polymer (MIP) modified carbon paste electrode (CPE) was developed for electrochemical detection of L-cysteine (Cys). Characterisation of MIP was done with FTIR and the modified electrode with cyclic voltammetry (CV) and differential pulse voltammetry (DPV). CV, DPV and impedance analysis demonstrated that the modified electrode is responsive towards the target molecule. The optimum percentage composition of MIP for MIP/CPE and the effect of pH towards the electrode response for Cys were studied. The detection of Cys in the range of 2×10^{-8} to 18×10^{-8} M at MIP/CPE was monitored by DPV with a limit of detection of 9.6 nM and R^2 of 0.9974. Also, various physiological interferents such as ascorbic acid, L-tryptophan, D-glucose, D-cysteine and L-cysteine were found to have little effect on DPV response at MIP/CPE. The utility of the electrode was proved by the effective detection of Cys from tap water and human blood plasma samples with reproducible results.

1. Introduction

L-cysteine (Cys), a non-essential amino acid, plays a key role in biological systems especially in communication between the immune system cells and in a variety of important cellular functions such as protein synthesis, metabolism [1] and detoxification reactions [2]. Cys is considered as a 'paravitamin' (a substance resembling a vitamin), as it and its derivatives play a role similar to that of vitamins particularly antioxidative vitamins by controlling the oxidant/antioxidant balance and by indirectly regulating the metabolic processes. Unlike vitamins, Cys serves as a building unit of proteins and as a source of energy [3]. The altered levels of Cys have been implicated in a number of clinical situations such as cervical cancer [4], Alzheimer's and Parkinson's disease [5] and pathological conditions including HIV (human immunodeficiency virus) [6]. Hence, Cys possesses high significance in medicine and food chemistry due to its bioactivity and therefore has pharmaceutical applications as antibiotics, cancer indicator etc.

Cys determination has been reported using spectrophotometry [7,8], fluorimetry [9], chemiluminescence [10–12], HPLC [13–15] etc. However, most of these are composed of technical hitches such as sample preparation, necessity of molecules derivatisation or lack of sufficient sensitivity that limit their utility [16]. Compared to these, electrochemical analysis presents advantages such as simplicity, high sensitivity and low cost, but a major problem related to the thiol detection is the high overpotential required for the most conventional electrodes [17]. But it also provides only low selectivity and reproducibility, which could be rectified by the use of chemically modifying the electrodes with a species having inherent selectivity such as molecularly imprinted polymers (MIP). MIPs are synthesised by polymerising the monomers which are bonded covalently/non-covalently to the target molecule (or template) followed by leaching out the template. The use of electrochemical methods incorporating MIPs is a well established analytical technique employing the concept of selective uptake of an analyte of interest and subsequent generation of a characteristic electrochemical signal [18,19]. Recently several MIP based electrochemical sensors were reported for dopamine [20], chloramphenicol [21], and folic acid [22].

Herein, a molecularly imprinted polymer of methacrylic acid modified carbon paste electrode was developed, characterised by various electroanalytical techniques and was used for the electrochemical detection of Cys by differential pulse voltammetry and impedance using $K_4[Fe(CN)_6]^{3-/4-}$ as a marker molecule.

2. Experimental

2.1. Chemicals and apparatus

L-cysteine (Cys), methacrylic acid (MAA), ethyleneglycoldimethacrylate (EGDMA), 2,2′-azobis(2-methylpropionitrile) (AIBN), tetrabutylammonium perchlorate (TBAP), D-glucose and D-cysteine from Sigma-Aldrich, graphite and L-ascorbic acid from Alfa Aesar, methanol, acetic acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate and potassium chloride from Merck, sodium hydroxide, potassium ferrocyanide and potassium ferricyanide from Qualigens and L-tryptophan and L-cystine from Loba Chemie were used as obtained. All chemicals used were of analytical reagent grade and all solutions were prepared in ultrapure water (Millipore-Q). All the solutions used for electrochemical

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http://dx.doi.org/10.1016/j.msec.2014.01.020

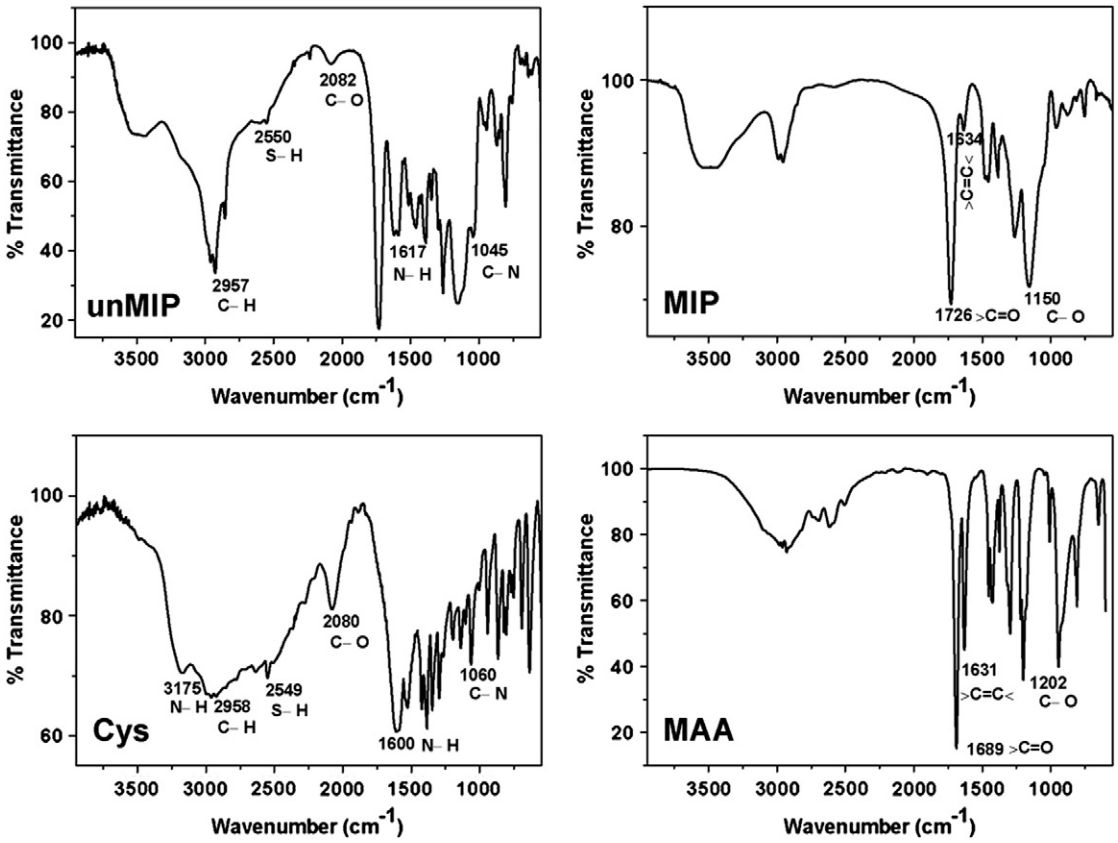

Fig. 1. FTIR of MAA, Cys, unMIP and MIP.

analysis were prepared in 0.1 M phosphate buffer solution (PBS) of pH 7 and freshly prepared stock solution of 1 mM Cys was used throughout the studies.

A EUTECH make Cyberscan pH meter was used for adjusting pH of buffer solutions and a centrifuge apparatus of Remi make (PR 24) for centrifugation. Thermo Scientific Nicolet iS5 FTIR Spectrometer was employed for recording the Fourier transform infrared (FTIR) spectra. An electrochemical workstation (CHI6043B, CH instruments, USA) coupled with a three-electrode cell was used for cyclic voltammetric (CV), differential pulse voltammetric (DPV) and electrochemical impedance studies. Different modified carbon paste electrodes (CPE) of 3 mm diameter, a Pt wire and a saturated Ag/AgCl electrode were used as the working electrode, counter electrode and the reference electrode, respectively.

2.2. Procedure

2.2.1. Preparation of imprinted polymers

The molecularly imprinted polymer (MIP) was prepared aiming Cys as the target molecule. Cys (1 mM) and MAA (4 mM) were dissolved in 60 ml of methanol and were stirred for 12 h. EGDMA (20 mM) and AIBN (1 mM) were added into it, followed by 15 min nitrogen purging in a sealed atmosphere. It was allowed to polymerise at 60 °C under a constant 24 h stirring and the produced Cys containing MIP or unleached

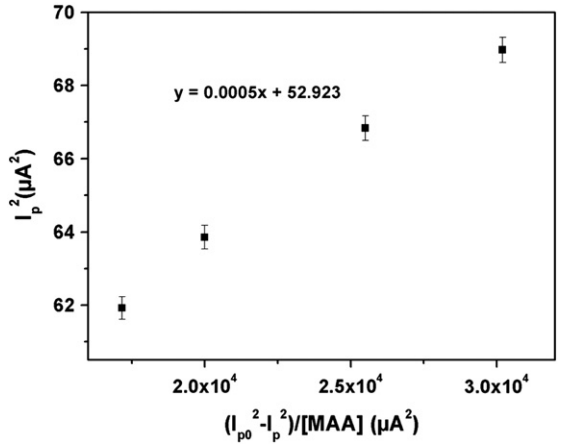

Fig. 2. Plot of I_p^2 vs. $(I_{po}^2 - I_p^2)/[MAA]$ for 1 mM Cys with varying concentrations of MAA ranging from 0.2 to 0.8 mM with an increment of 0.2 mM in a medium buffered at pH 7.

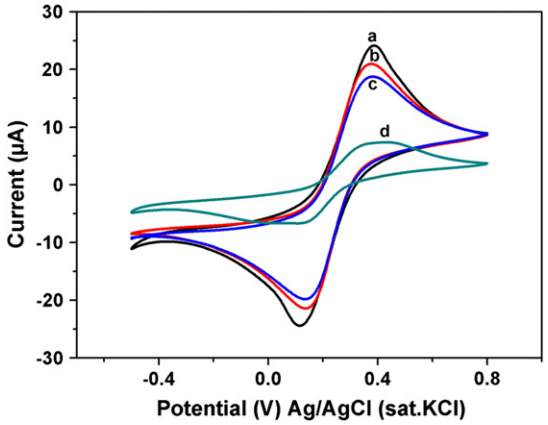

Fig. 3. CV analysis of (a) bare CPE, (b) MIP/CPE, (c) MIP/CPE after interaction with 1 mM Cys for 10 min and (d) NIP/CPE conducted in 10 mM $K_4[Fe(CN)_6]^{3-/4-}$ having 1 M KCl prepared in 0.1 M PBS.