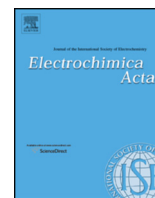




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Functionalized multiwalled carbon nanotubes-nanostructured conducting polymer composite modified electrode for the sensitive determination of uricase inhibitor[☆]

K. Rajalakshmi, S. Abraham John^{*}

Centre for Nanoscience and Nanotechnology, Department of Chemistry, Gandhigram Rural Institute, Gandhigram – 624 302, Dindigul, India

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ABSTRACT

The acid functionalized-multiwalled carbon nanotubes (FMWCNTs)-nanostructured 5-amino-2-mercapto-1,3,4-thiadiazole (AMT) composite film was fabricated on glassy carbon electrode (GCE) for the sensitive determination of rutin (Rut). The composite film was characterized by SEM, FT-IR, attenuated total reflectance (ATR)-FT-IR, XPS and cyclic voltammetry. The attachment of FMWCNTs on the electrode surface through amide bond formation was confirmed from the C1s peak at 287.3 eV in XPS and the band at 1681 cm⁻¹ in ATR-FT-IR spectroscopy. The FMWCNTs-AMT composite film was then exploited for the determination of uricase inhibitor, rutin (Rut) in 0.2 M phosphate buffer solution (pH 3). The oxidation of Rut at composite electrode occurs with 30 mV less positive potential and 3.2-fold higher oxidation current when compared to bare GCE. The amperometric current response was increased linearly when Rut concentration was increased from 10–1000 nM and the detection limit was found to be 0.3 nM (S/N=3). Further, the composite modified electrode successfully determined the concentration of Rut in the presence of 250-fold higher concentration of one of the major interferences, ascorbic acid. The practical application of the present modified electrode was demonstrated by determining the concentration of Rut in tablets.

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1. Introduction

Rutin (3',4',5,7-tetrahydroxyflavone-3 β -D-rutinoside (Chart 1), Rut) is a kind of flavonoid glycoside and naturally occurs in the plants such as *Flos Sophorae*, *Sophora Japonica* and also in common human diet [1]. The daily intake of flavonols plus flavones has been estimated as 3–80 mg [2]. Rut has anti-inflammatory [3], anti-oxidant [4], anti-progestational [5], anti-tumour [6], anti-viral, anti-allergic and anti-bacterial [7,8] properties. It involves in the direct action on the capillary bed and decreases the permeability and fragility of the blood vessels [9]. Besides, it shows radical scavenging activity and metal chelating properties [9]. It involves osteoclast formation induced by receptor activator of NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) ligand in bone marrow-derived macrophages [10]. The high dietary intake of Rut from fruits, vegetables, tea and red wine may

be associated with the reduced risk from coronary heart diseases [11]. It exerts stronger production against nitrosative stress and hepatocellular damage [12]. Along with allopurinol and quercetin, it increased the urinary uric acid/creatinine ratio [13]. Rut reduced serum uric acid levels in hyperuricemic rodents induced by uricase inhibitor potassium oxonate via the inhibition of hepatic xanthine oxidase activity [14]. Hence, it is used as a therapeutic medicine and various preparations containing Rut are registered as drugs worldwide [15]. Even though, it has several medicinal properties, the appropriate amount of Rut can elevate the antioxidant status in liver without decreasing zinc, copper and iron in the brain and liver [16]. However, the excessive intake of Rut may cause a decrease in body weight and loss of zinc, copper and iron. Therefore, a sensitive and accurate analytical method is essential for the determination of Rut. It has been reported that Rut administered with ascorbic acid (AA) showed better results than the use of Rut alone [1]. Therefore, it is also important to determine the concentration of Rut in the presence of AA.

Several techniques are available in the literature for the determination of Rut including high performance liquid chromatography [17], capillary electrophoresis [18], spectrophotometry [19], sequential injection analysis [20], resonance light scattering [21] and electrochemical [22–26]. Among these, electrochemical

[☆] This paper is dedicated to Professor Takeo Ohsaka, Tokyo Institute of Technology- Nagatsuta on his retirement and his pioneering works in the fields of electroanalytical chemistry, bioelectrochemistry, batteries and materials chemistry.

^{*} Corresponding author. Tel: +91 451 245 2371; Fax: +91 451 245 3031

E-mail addresses: abrajohn@yahoo.co.in, s.abrahamjohn@ruraluniv.ac.in (S. A. John).

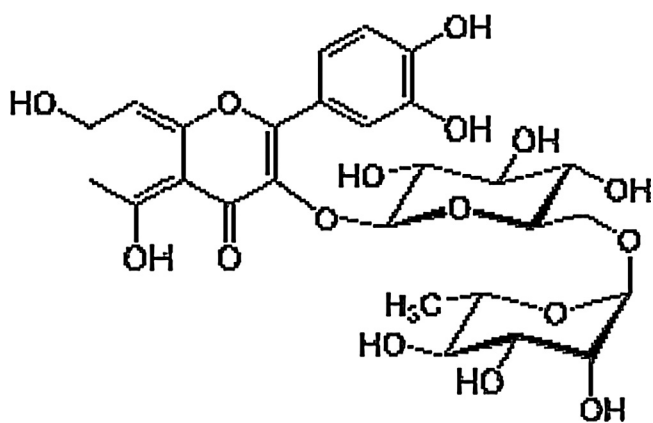


Chart 1. Chemical structure of Rut.

method has several advantages which include simple operation, inexpensive, fast response, less time consuming, high sensitivity and selectivity compared to other methods.

Nowadays, application of carbon nanotubes (CNTs) for the fabrication of electrochemical sensors has received much attention [27,28] mainly due to their high electrical conductivity [29]. Recently, our research group has studied the application of nanostructured heteroaromatic film modified electrodes for the determination of several biologically important molecules [30,31]. It has been already demonstrated that CNTs-polymer composite modified electrode enhanced the electrochemical sensing properties when compared to individual CNTs or polymer modified electrode alone [32]. Carbon based materials and their composites have been already used for the determination of Rut [22–24,27]. However, the modification of these electrodes involves several steps and tedious procedures. For example, Yang et al. reported the determination of Rut using gold nanoparticles/ethylenediamine/carbon nanotube modified glassy carbon electrode. In this paper, the electrode is first modified by drop casting methanolic solution of Nafion-FMWCNTs, followed by electrochemical grafting of ethylenediamine and finally citrate-capped gold nanoparticles are self-assembled on the electrode surface [24]. The objective of the present work is to fabricate functionalized multiwalled carbon nanotubes (FMWCNTs)-nanostructured conducting heterocyclic film composite modified electrode in two steps and utilize the electrode for the highly sensitive determination of Rut.

In the present study, FMWCNTs-conducting film composite electrode was prepared by covalent attachment of acid FMWCNTs

on glassy carbon electrode (GCE) using 1,8-octanediamine linker and dicyclohexylcarbodiimide (DCC) coupling agent. Then, 5-amino-2-mercapto-1,3,4-thiadiazole (AMT) monomer was electrochemically polymerized on the surface of the FMWCNTs modified GCE. The modified electrodes were characterized by SEM, XPS and cyclic voltammetry. Then, the FMWCNTs/p-AMT modified electrode was used for the determination of Rut. It was found that the composite modified electrode not only shifted Rut oxidation potential towards less positive potential but also enhanced its oxidation current 3.2-fold when compared to bare GCE. Further, the oxidation current of Rut increases linearly while increasing the concentration of Rut from 10–1000 nM and the limit of detection was found to be 0.3 nM ($S/N=3$).

2. Experimental details

2.1. Chemicals

Na_2HPO_4 and NaH_2PO_4 were used to prepare the phosphate buffer (PB) solution (pH 3) with double distilled water by adjusting with ortho-phosphoric acid. 5-amino-2-mercapto-1,3,4-thiadiazole (AMT), acid functionalized multiwalled carbon nanotubes (FMWCNTs), dicyclohexylcarbodiimide (DCC), 1,8-octanediamine (OD) and ascorbic acid (AA) were purchased from Aldrich and were used as received. Rutin (Rut) and GC plates were purchased from Alfa Aesar. For taking SEM, XPS and ATR-FT-IR analysis GC plates were used. All other chemicals used in this investigation were of analytical grade.

2.2. Instrumentation

Electrochemical measurements were performed in a conventional two-compartment three electrode cell with a mirror polished 3 mm GCE as a working electrode, Pt wire as a counter electrode and NaCl saturated Ag/AgCl as a reference electrode. The electrochemical measurements were carried out with a CHI model 634B electrochemical workstation (CH Instruments, Austin, TX, USA). XPS measurements were carried out by Shimadzu Axis 165 high performance multitechnique analysis using an Al $K\alpha$ source with pass energy of 80 eV, where the dwell time was 458 ms and the pressure in the analysis chamber was lower than 133.3×10^{-8} Pa. The binding energies for identical samples were reproducible within ± 0.10 eV. A survey spectrum and core-level spectra of C1s (282–292 eV) region was systematically recorded. XPS spectra were analysed using XPSPEAK41 software. After subtraction of a Shirley background, all spectra were fit by using

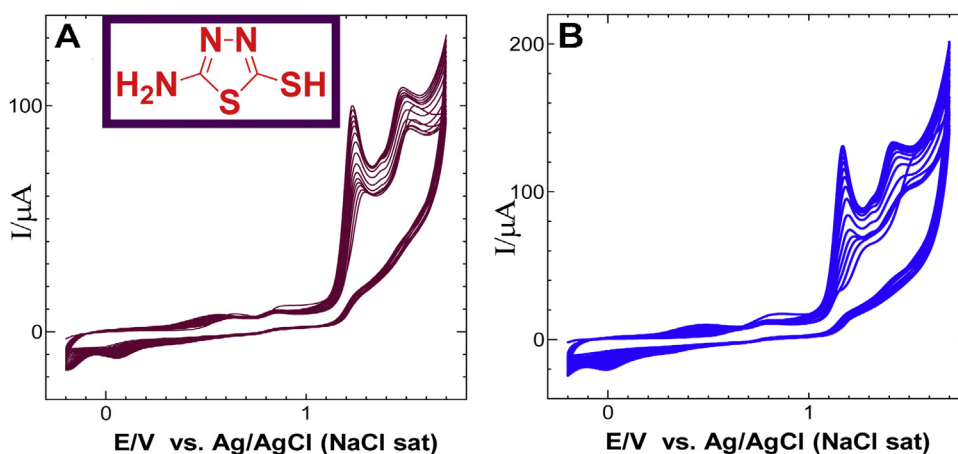


Fig. 1. Electrochemical polymerization of AMT (15 cycles) at (a) bare GC and (b) GCE/OD/FMWCNTs in 1 mM AMT containing 0.1 M H_2SO_4 at a scan rate of 50 mV s^{-1} . Inset: Structure of AMT molecule.

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