



Sensitive electrochemical sensor for simultaneous determination of uric acid and xanthine in human biological fluids based on the nano-boron doped ceria modified glassy carbon paste electrode



Hossieny Ibrahim*, Yassien Temerk*

Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt

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ABSTRACT

A sensitive and selective electrochemical method was developed for simultaneous determination of uric acid and xanthine using the nano boron doped ceria modified glassy carbon paste electrode (nano-B-CeO₂/GCPE). The proposed sensor exhibited an excellent catalytic activity, high sensitivity and good selectivity toward the oxidation of uric acid and xanthine. The potential mutual interference of uric acid (UA), xanthine (XA), dopamine (DA) and ascorbic acid (AA) were examined indicating that their oxidation take place independently at the nano-B-CeO₂/GCPE sensor. For simultaneous detection by synchronous change of the concentration of UA and XA, the linear ranges were 0.42–11.87 μM and 0.07–2.02 μM with detection limits of 5.39×10^{-9} M and 2.36×10^{-9} M, respectively. The proposed method was successfully applied to simultaneous determination of UA and XA in human urine and serum samples with satisfactory results.

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1. Introduction

Uric acid (UA) and xanthine (XA) are degradation products of purine metabolism in human beings and higher primate [1]. UA is a product of the metabolic breakdown of purine nucleotides and the abnormal level of UA results in several diseases like gout, hyperuricaemia and pneumonia [2,3]. XA is an important intermediate of purine metabolism and the abnormality of XA leads to xanthinuria and its therapeutic level is 10–20 mgmL⁻¹ in blood [4]. Quantification of UA and XA has been of the utmost importance in the recent years as they usually coexist in extracellular fluids and play an important role in maintaining physiological homeostasis of organisms [5]. Since several physiological properties are related to the concentrations of these two structurally similar purine derivatives, simultaneous determination of them is very important in the clinical point of view. Hence, in order to study their physiological function and aid clinical diagnosis at early stages of related diseases, it is necessary to develop methods for simultaneous determination of UA and XA in biological samples.

Various techniques have been developed to simultaneously determine the purine degradation products such as enzymatic methods [6], high performance liquid chromatography (HPLC) [7,8], capillary

electrophoresis (CE) [9,10] and electrochemistry [11–40]. In comparison with other methods, electrochemical methods showed some advantages, including higher selectivity and sensitivity. However, voltammetric UA and XA sensing typically suffers from interference from AA and DA and these substances show similar electrochemical behaviours and interfere each other. Hence, AA and DA have been to be taken into account in the case of electrochemical determination of UA and XA simultaneously in physiological fluids.

In recent years, chemically modified electrodes have attracted large interest due to their potential applications in various analysis. Modifying the working electrode with metal and metal oxide particles is the most widely employed nanomaterials owing to excellent physical and catalytic properties of those materials [41]. In this context, rare earth based nanomaterials are a technologically important group of materials and have attracted enormous attention because of their advanced electronic, optical and catalytic properties [42]. Ceria and ceria based materials are important oxidation catalysts as a result of their significant oxygen storage capability (OSC) and inherent Ce³⁺/Ce⁴⁺ redox cyclic and are commonly used as the oxide supports in automotive three way catalysts (TWCs) to decrease the toxic pollutant exhaust gases [43]. Recently, metal-doped ceria nanomaterials have been widely considered to promote the active oxygen content on the CeO₂ surface by changing the surface element composition, because such materials ultimately reveal strikingly high catalytic activity [44]. In this case, it was assumed that the overall conductivity of doped oxide ceramics turns from ionic into electronic upon grain size reduction into nanometric

* Corresponding authors.

E-mail addresses: Hossienyomer@yahoo.com, hossieny.ibrahim@aun.edu.eg (H. Ibrahim), Temerk44@yahoo.com, temerk@aun.edu.eg (Y. Temerk).

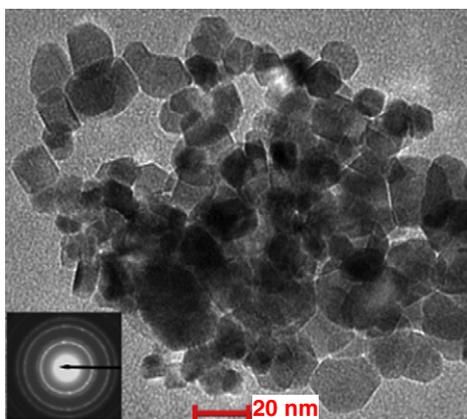


Fig. 1. TEM image of 10% B doped nano-CeO₂ (inset is SAED pattern).

range [45]. Different strategies are adopted the physico-chemical properties and morphology of the nanostructured CeO₂ in order to achieve high sensitivity and selectivity. In this context, boron is the most efficient dopant to improve and tune the electrical, optical and magnetic properties of CeO₂ [46]. The substitution of Ce⁴⁺ (0.97 Å) ions by B³⁺ (0.23 Å) creates more oxygen vacancies in the CeO₂ system leading to a decrease in the free electron concentration and an optimal doping level is expected to improve the catalytic activity of CeO₂. Hence, the application of the nano-B-CeO₂ into glassy carbon paste as a modifier seems to improve the performance of the electrode toward the oxidation of UA and XA with enhanced voltammetric responses and well resolved peaks for individual analytes in the mixed system. Modifying the GCPE with suitable catalyst materials such as nanocrystalline B-CeO₂ provides an additional advantage to obtain well separated peaks and enhanced signals for simultaneous detection of UA and XA in the presence of ascorbic acid and dopamine which coexist with the investigated purine derivatives in biological fluids.

In the present work, we described a novel strategy for the simultaneous electrochemical determination of UA and XA by using the nano-B-CeO₂/GCPE. Due to facile electron transfer reaction at the nano-B-CeO₂, the modified electrode separates the voltammetric signals of UA and XA with enhanced peak current compared to bare GCPE and undoped CeO₂/GCPE. Interestingly, in this work, the potential mutual interferences of UA, XA, AA and DA were examined suggesting that their oxidation take place independently at the nano-B-CeO₂/GCPE sensor. The practical application of the present modified electrode was demonstrated by simultaneously determining the concentrations of UA and XA in human blood serum and urine samples without cross interference of AA and DA.

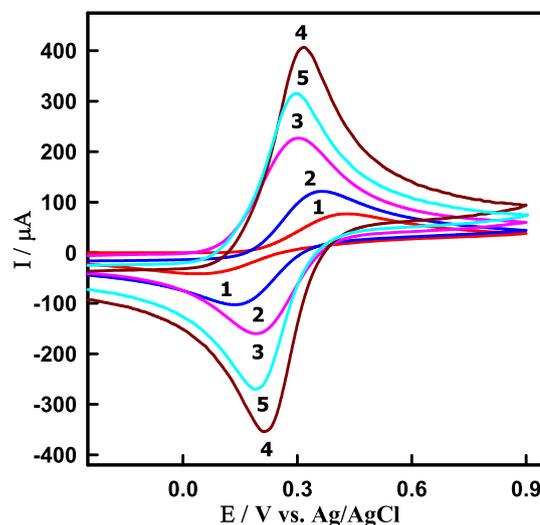


Fig. 3. Cyclic voltammograms of 5 mM [Fe(CN)₆]^{3-/4-} in 0.1 M KCl obtained at a scan rate of 100 mV/s. Curve 1: bare GCPE, curve 2: undoped nano-CeO₂/GCPE, curve 3: 5%, curve 4: 10% and curve 5: 15% nano-B-CeO₂/GCPE.

2. Experimental

2.1. Reagents

UA, XA, DA, AA, boric acid, cerium(III) nitrate hydrate and polyvinyl alcohol polymer (PVA) (MW = 30,000 g/mol), were purchased from Sigma-Aldrich chemicals (St. Louis, Mo, USA). Glassy carbon spherical microparticles with a diameter of 0.4–12 μm and mineral oil were obtained from Alfa Aesar (Ward Hill, MA). Freshly prepared solutions of UA, XA, DA and AA were used in all experiments. Phosphate buffer solutions (PBS, 0.2 M) with different pH values were prepared from analytical grade chemicals (NaH₂PO₄ and Na₂HPO₄, from Merck, Darmstadt, Germany) by mixing stock solutions of 0.2 M NaH₂PO₄ and 0.2 M Na₂HPO₄, and the pH adjusted with 0.1 M H₃PO₄ or NaOH solution. The pH values of the buffer solutions were measured with a digital radiometer pH meter, Jenway 3310 accurate to ±0.02 unit. Ultrapure water (Milli-pore Inc., 18.2 MΩ cm) was obtained from a Milli-Q purification system and used in all experiments.

2.2. Instrumentation

Electrochemical experiments including cyclic voltammetry (CV) and square-wave voltammetry (SWV) were carried out using an EG&G PAR 384 B (Princeton Applied Research, Oak Ridge, TN, USA) polarographic analyzer controlled by 394 software in conjunction with a PAR Model

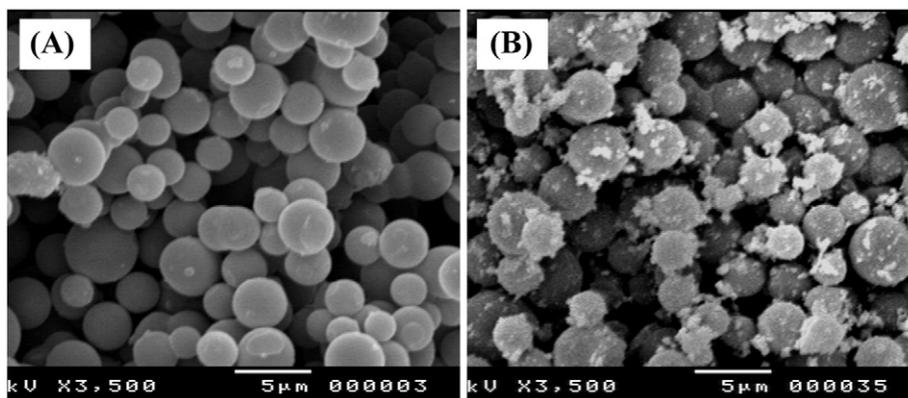


Fig. 2. SEM images of (A) GCPE and (B) 10% B doped nano-CeO₂/GCPE.

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