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Simultaneous determination of uric acid, xanthine, hypoxanthine and caffeine in human blood serum and urine samples using electrochemically reduced graphene oxide modified electrode

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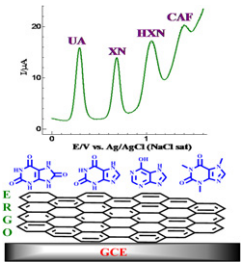
HIGHLIGHTS

- Glassy carbon electrode was modified with graphene by self assembly method.
- Modified electrode was characterized by Raman spectroscopy.
- Graphene modified electrode separated the voltammetric signals of four purine derivatives.
- Selective and simultaneous determination of four purine compounds were achieved.
- Practical application was demonstrated in human blood serum and urine samples.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper describes the fabrication of graphene on glassy carbon electrode (GCE) by electrochemical reduction of graphene oxide (GO) attached through 1,6-hexadiamine on GCE and the simultaneous determination of structurally similar four purine derivatives using the resultant electrochemically reduced GO (ERGO) modified electrode. The electrocatalytic activity of ERGO was investigated toward the oxidation of four important purine derivatives, uric acid (UA), xanthine (XN), hypoxanthine (HXN) and caffeine (CAF) at physiological pH. The modified electrode not only enhanced the oxidation currents of the four purine derivatives but also shifted their oxidation potentials toward less positive potentials in contrast to bare GCE. Further, it successfully separates the voltammetric signals of the four purine derivatives in a mixture and hence used for the simultaneous determination of them. Selective determination of one purine derivative in the presence of low concentrations other three purine derivatives was also realized at the present modified electrode. Using differential pulse voltammetry, detection limits of 8.8×10^{-8} M, 1.1×10^{-7} M, 3.2×10^{-7} M and 4.3×10^{-7} M were obtained for UA, XN, HXN and CAF, respectively. The practical application of the modified electrode was demonstrated by simultaneously determining the concentrations of UA, XN, HXN and CAF in human blood plasma and urine samples.

1. Introduction

Purine bases and their derivatives play an important role in the functioning of living systems [1]. Purines are involved in many metabolic processes as cofactors which play key roles in biological fundamental processes [2]. Several pathological conditions alter the levels of purine derivatives in blood serum and urine which are

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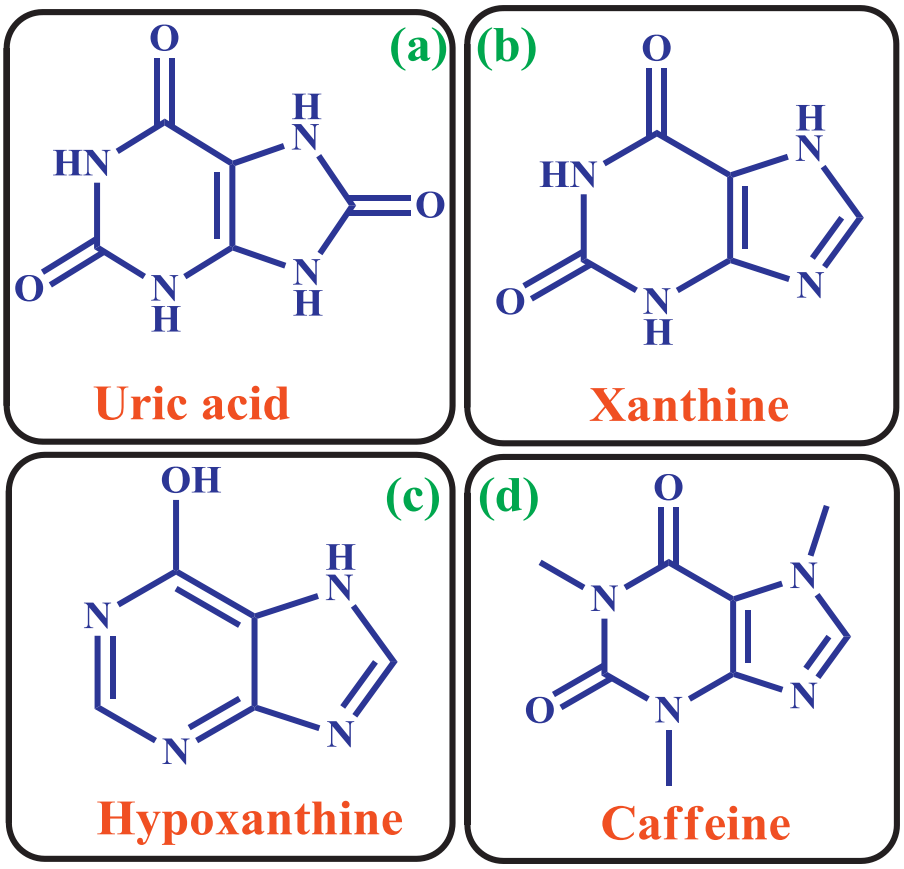

Chart 1. Structures of purine derivatives.

biochemical indicators for several diseases [2]. The levels of uric acid (UA), oxypurines such as xanthine (XN) and hypoxanthine (HXN) and trimethylxanthine derivative, caffeine (CAF) (Chart 1) may provide sensitive indicators for physiological diseases [3,4]. UA, XN and HXN are present in body fluids by the metabolism of purine derivatives whereas CAF is introduced into the body fluids by consuming tea, coffee and coca cola [5]. 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 [6]. In normal serum, UA levels are less than 420 μmol L^{-1} in men and 330–360 μM L^{-1} for women [7]. XN is an important intermediate of the purine metabolism and the abnormality of XN leads to xanthinuria and its therapeutic level is 10–20 mg mL^{-1} in blood [8]. HXN is found as a minor purine base in tRNA and also a product in the metabolism of purine nucleotides [9]. It is an important factor to know the fish freshness [10]. CAF, a methyl derivative of xanthine, is a drug which has many physiological effects such as stimulant to central nervous system and cardiac muscles, dieresis and positive effect on cardiovascular system [11]. Several epidemiological studies show that CAF consumption and plasma UA level were related to the incidence of some neurodegenerative diseases [12,13]. Determination of XN and UA levels is important for diagnosing gout and hyperuricaemia [14]. Since several physiological properties are related to the concentrations of these four structurally similar purine derivatives, simultaneous determination of them is very important in the clinical point of view. Several reports were available in the literature for the simultaneous determination of UA, XN and HXN [15–23] and individual determination of CAF [24–29]. However, to the best of our knowledge, no report is available in the literature for the simultaneous determination of UA, XN, HXN and CAF. Hence, the objective of the present study is to simultaneously determine the four purine derivatives using the graphene modified glassy carbon electrode (GCE).

Among the different allotropes of carbon, graphene receives huge attention due to its unique thermal, electrical and mechanical properties which originate from the two dimensional, single atom thick structure of graphene [30,31]. The unique properties of graphene make them to be used in supercapacitors [32], fuel and solar cells [33] and biosensors [34,35]. The high specific surface area, besides high conducting nature of graphene layers, makes them to be used as a novel material for the fabrication of electrodes [36]. Recently, researchers use graphene to fabricate electrodes for the electrochemical sensing of metal ions [37], biomolecules [38] and toxic chemicals [39]. Most of the researchers use drop casting method for the fabrication of graphene on different electrode substrates [40–43]. Self-assembly is the best method to fabricate graphene films with desirable thickness on electrode substrates [44]. In the present study, the precursor of graphene, graphene oxide (GO), was electrostatically assembled on GCE through diamine linker. The electrostatically assembled GO was electrochemically reduced at more negative potential to retain the aromatic backbone of graphene and used for the electrochemical sensing of structurally similar purine derivatives. Due to facile electron transfer reaction at ERGO films, the modified electrode separates the voltammetric signals of the four analytes with enhanced peak currents compared to bare GCE and also prevents the surface fouling effect caused by the oxidation products of purine derivatives. The practical application of the present modified electrode was demonstrated by simultaneously determining the concentrations of UA, XN, HXN and CAF in human blood serum and urine samples.