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A fluorescent biosensor for the determination of xanthine in tea and coffee via enzymatically generated uric acid

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ABSTRACT

Herein, the use of rodamine B capped-thioglycolic acid functionalized gold nanoparticles (RB-capped TGA/GNPs) as a novel fluorescence probe for the sensitive determination of xanthine (XA) has been demonstrated. The sensing mechanism is based on the enzymatically generated uric acid (UA) induced degradation of gold nanoparticles which resulted in the quenching of its fluorescence. By virtue of the specific response, the present assay allowed for the selective determination of XA in the range of 93 nmol/L-0.84 μmol/L with a detection limit of 10.1 nmol/L and that of UA in the range 9.90 nmol/L-65.4 nmol/L with the detection limit being 9.71 nmol/L. In addition, the application of the present approach in real samples like tea and coffee and also in synthetic blood serum has been demonstrated.

1. Introduction

XA and UA are the degradation products of purine catabolism in human beings and higher primates (Tiliang, Xiangying, & Bin, 2011). XA is the intermediate of purine degradation metabolism and UA-the final oxidation product (Yan & Li-li, 2010). The two products can enter the cell membranes and cumulate in extracellular fluids (Yan & Li-li, 2010). Depending on the concentration level of these purine metabolites in body fluids such as urine and human serum, these can act as markers for many clinical conditions, including perinatal asphyxia, xanthinuria, leukemia, hyperuricemia and renal failure, and so the accurate detection and quantification of XA and UA in body fluids are extremely important in study of the homeostasis of the body and clinical diagnosis (Yan & Li-li, 2010; Pu, Huawen, Liping, & Xianying, 2015; Yun & Jinzhou, 2012). XA is also a significant biomarker in food industries (Pu et al., 2015). Numerous mild stimulants such as caffeine and theobromine which have been derived from XA are present in coffee and tea (Devi, Narang, Yadav, & Pundir, 2012). Recently there has been many reports on the electrochemical determination of XA based on xanthine oxidase (XOD) catalysed reaction forming hydrogen peroxide (H_2O_2) (Yun & Jinzhou, 2012; Arslan, Yasar, & Kilic, 2006; Sun et al., 2009; Devi, Yadav, & Pundir, 2011; Wang & Tong, 2010). Although these method contributes greatly to XA detection, most of them are time consuming and laborious procedures. In such a scenario, the development of simple and sensitive method for XA sensing and in-turn the determination of UA becomes significant. There are also reports of several other techniques for the determination of XA and UA including spectrophotometry (Galbán, Andreu, Almenara, de Marcos, & Castillo, 2001; Pu et al., 2015), high performance liquid chromatography (HPLC) (Rebelo, Piedade, & Oliveira-Brett, 2004; Thomas et al., 2013), fluorometric mass spectrometry and fragmentography (Olojoa, Xiab, & Abramsona, 2005), capillary electrophoresis (Munoz, Lopez-Mesas, & Valiente, 2010) etc. These techniques have problems that limit sensitivity and specificity of the probe. The major drawback of the capillary electrophoresis method with UV detection is its poor sensitivity, which limits its application to the determination of trace uric acid in some biological samples. HPLC often require tedious sample purification steps to remove potentially interfering chemicals in the matrices.

Colorimetric sensors have been recently emerging for the determination of xanthine. For example (Pu et al., 2015), have reported a colorimetric method for XA based on the aggregation of gold nanoparticles wherein XA induces gold nanoparticles (GNPs) aggregation through hydrogen-bonding interaction. As a

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consequence, the color of the solution changes from red to blue, in correlation with the concentration of XA. Although the work is fast and easy to perform, it cannot be used for the detection of both XA and its oxidation product UA.

Both XA and uric acid are present in body fluids. The concentration of XA in blood and urine is lower than UA (Jesny & Girish Kumar, 2017). Their concentrations in these body fluids are increased in individuals who frequently consume a high amount of sugar-sweetened soft drinks and beverages like tea, coffee and alcohol (Ngoc et al., 2010). Since several physiological properties are related to the concentrations of these two structurally similar purine derivatives, simultaneous determination of these is very important in the clinical point of view.

In this work, a fluorescent sensor has been developed for the determination of XA via its oxidation product UA. It is found that the fluorescence of RB-capped TGA/GNPs can sensitively respond toward the concentration of UA. Moreover, XA can be oxidized to UA in the presence of XOD via the following reaction (Urszula, 2012; Xian-Xiang, Qi, Zhi, & Qian-Ming, 2011).

$$XA + H_2O + O_2 \rightarrow UA + H_2O_2;$$

and the generated UA leads to the aggregation of GNPs which could be visually detected from the color change. Here, XA can be detected by the amount of the enzymatically generated UA. Therefore, a sensitive and cost-effective fluorescent sensor for the determination of XA via UA has been fabricated. Although there are reports for the electrochemical determination of these purine metabolites, determination of XA via UA using fluorescence technique has not been reported hitherto; to the best of our knowledge.

2. Experimental

2.1. Chemicals and instrumentation

All the reagents used were of analytical grade. XA, UA, adenine (Ade), guanine (Gua), ascorbic acid (AA), cholesterol (Ch), glucose (Glc), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$) and rodamine 6G were purchased from s.d.fine Chemicals, India; caffeine, theophylline and hypoxanthine from SRL, India. XOD from bovine milk (≥0.4 units/mg protein; enzyme activity being 1.66×10^{-8} kat) was purchased from Sigma-Aldrich and $HAuCl_4.3H_2O$, TGA from SPECTROCHEM, India. All solutions were prepared using Milli Q water. Absorption studies were performed on a UV-3500 Labomed Inc. spectrophotometer and fluorescence measurements were made using a JAZ-EL-200-X. Morphology of the nanoparticles was studied by Transmission emission microscopy (TEM) (JEOL/JEM 2100). Horiba Fluorolog-3 was used for lifetime measurements. Mass spectral data were obtained by direct injection to Waters 3100 mass detector with an electron spray ionisation unit.

2.2. Preparation of RB-capped TGA/GNPs

The synthesis of probe was carried out according to a reported procedure with slight modification (AiFang et al., 2009). Citrate capped gold nanoparticles were prepared by reduction and these were further modified with TGA through Au-S bonding. TGA-functionalized GNPs were obtained by adding 20 μL, 0.01 mol/L TGA to 10 mL of citrate capped GNPs solution under vigorous stirring. After reaction for 1 h at room temperature, TGA functionalized GNPs were obtained. To it, an aliquot of RB solution (200 μmol/L, 20 μL) was added. The adsorption of RB molecules on the surface of GNPs was complete after 15 min equilibration at room temperature.

2.3. Analytical procedure

10 μL of XOD (10 mg/mL) and 100 μL of XA (of various concentrations) in 0.01 mol/L sodium acetate buffer (pH 7.0) were incubated at 30 °C for 20 min. Then, 2 mL of RB-capped TGA/GNPs solution was added into the above XA reaction solution. The mixture was incubated in a 40 °C water bath for 5 min and fluorescence measurements were made by exciting at a wavelength of 400 nm. For the determination of UA, 2 mL of the probe was mixed with various concentrations of UA and fluorescence measurements were made. Fluorescence emission was scanned from 520 to 625 nm at room temperature. Linear graphs were obtained by plotting I_0/I vs. concentration of analyte with I_0 and I being the fluorescence intensities in the absence and presence of the analyte, respectively. The proposed method was applied to the determination of XA in real samples such as coffee and tea and in synthetic blood serum.

2.4. Preparation of samples

Commercially available tea/coffee powder (5.0 g) was boiled in 10 mL of distilled water for 10 min and filtered (Devi et al., 2012). The filtrate was then cooled to room temperature. XA content in coffee and tea extract was determined by the proposed biosensor in a similar way as described for the determination of XA under its ideal working conditions except that XA solution was replaced by coffee or tea extract.

The application of the proposed method was also studied in synthetic blood serum by spiking known concentrations of XA to it. Synthetic blood serum or ringer serum, a solution resembling blood serum in its salt constituents, was prepared in lab as per the procedure reported elsewhere (http://medical-dictionary.thefreedictionary.comRinger+lactate, 2017; Menon, Jose, Jesny, & Girish Kumar, 2016).

3. Results and discussion

3.1. Spectral characteristics of RB-capped TGA/GNPs

Fig. 1 shows the fluorescence spectrum of free unbound state RB molecules and the RB capped TGA/GNPs based sensor in the absence and presence of XA-XOD system. It was found that RB molecules in an unbound state exhibited a strong fluorescence

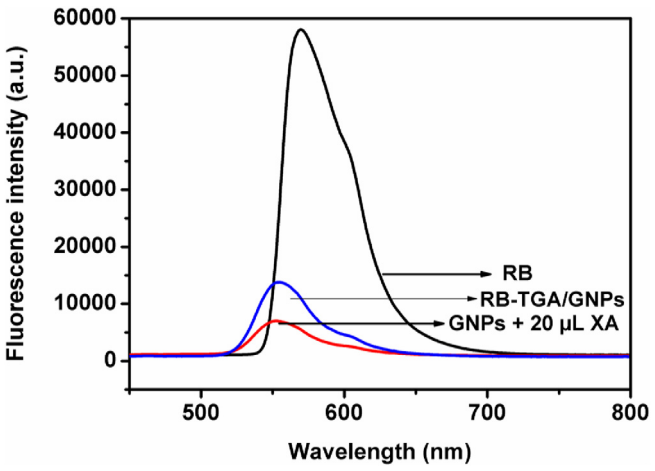

Fig. 1. Fluorescence spectra of solutions of Rodamine-B (2.0 μmol/L) and rodamine B capped-thioglycolic acid functionalized gold nanoparticles in the absence and presence of xanthine-xanthine oxidase system (xanthine; 93 nmol/L).