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# Study on detection methods for xanthine in food and biological samples

**Suhuan Wu, Siyue Jia, Xiaodong Dong***

College of Medicine, Hebei University, Baoding 071000, China

*Corresponding Author: xddong@hbu.edu.cn

## Abstract

Xanthine is generated from guanine by guanine deaminase and hypoxanthine by xanthine oxidase. The determination of xanthine in meat indicates its freshness, while its level in serum/urine provides valuable information about diagnosis and medical management of certain metabolic disorders such as xanthinuria, hyperurecemia, gout and renal failure. Therefore, the rapid, sensitive, and selective detection of xanthine level is significant for food and pharmaceutical analysis, clinical diagnosis applications. In this article the studies of detection methods for xanthine in recent years are reviewed.

**Keywords:** xanthine; guanine; determination; detection; sensor.

## 1. Introduction

Xanthine (3,7-dihydro-purine-2,6-dione) is a precursor of uric acid. Elevated levels of xanthine in blood and urine samples are indicative of pathological conditions such as xanthinuria, gout, or renal failure [1-3]. Xanthine level is very low in serum, which is about 100-times lower than that of uric acid. The concentration of xanthine in urine is normally extremely low, because it is converted immediately touric acid, a normal end-product of purine metabolism [4-6]. Hence determination of xanthine in serum/urine is very important in the diagnosis and medical management. Xanthine has also attracted much attention as an indicator for monitoring the meat freshness, especially in fish, as after the death of a fish, ATP gets degraded into xanthine, which increases with storage of fishmeat. Freshness of the fish meat is essential in food and pharmaceutical industries for manufacturing of high quality products [7-9]. Therefore, the highly selective detection of xanthine is of great importance in pharmaceutical analysis, clinical diagnosis, and food industries. Many strategies have been reported for the determination of xanthine. In this paper, the attributes of different analytical technique for the determination of xanthine in recent years are reviewed.

## 2. Analytical Methods

**2.1. Colorimetric method.** Colorimetry has commonly been used for routine analysis due to its simplicity, low-cost and practicability. It does not require any expensive or sophisticated instruments and the color changes can be even directly observed by the naked eye. Recently, some colorimetric systems have been set up to detect various kinds of substances such as DNA, biologically relevant molecules, metal ions, viruses and micro-organisms and so on [10-12].

Over the last few decades, the gold nanoparticles (AuNPs) have been widely investigated and applied in molecular catalysis and biosensors due to their unique electrical and optical properties. Typically, the synthesized AuNPs with a size of 13 nm exhibit a specific absorption band around 520 nm while dispersed in liquid media. With the continual aggregation of AuNPs, the increased particle size will cause a red shift in the absorption spectrum which is easy to observe and analyze. Because of the high extinction coefficients and the unique size dependent optical property of AuNPs, AuNP-based colorimetric assays have been widely applied for detecting DNA, proteins, metal ions and small molecules. The reasonable designs of the surface

chemistry of AuNPs promote specific interactions between receptors and analytes, leading to a highly selective detection [13,14]. These methods require a very simple sample preparation process and minimal apparatus investment and can be conducted in the field with portable devices. Therefore, they are very promising in the field of sensor.

Pu *et al.* [15] described a highly sensitive method for the determination of traces of xanthine based on the aggregation of citrate-stabilized AuNPs. Under optimal conditions of pH, the imide group of xanthine was adsorbed on the surface of the AuNPs, thereby displacing citrate ions, which led to an aggregation of the AuNPs via hydrogen-bond interactions. As a result, the color of the solution changes from red to blue which could be seen with bare eyes and also could be measured by spectrophotometry. The ratio of the absorbances at 630 nm and 520 nm was linearly related to the concentration of xanthine in the 125 nM to 6.0 μM range, and the detection limit was 23 nM. The method was simple, fast and feasible.

Wang *et al.* [16] demonstrated that bovine serum albumin (BSA) stabilized Au clusters exhibited highly intrinsic peroxidase-like activity. Unlike nature enzymes, the BSA-Au clusters had strong robustness and could be used over a wide range of pH and temperature. Because of ultra-small size, good stability and high biocompatibility in water solution compared with other kinds of nanoparticles as peroxidase mimetics, such as $Fe_3O_4$, FeS or graphene oxide, it was more competent for bioanalysis. They developed a sensitive and selective method for xanthine detection using xanthine oxidase (XOD) and the as-prepared BSA-Au clusters. The detection limit of this assay for xanthine was $5\times10^{-7}$ M and the proposed method was successfully applied for the determination of xanthine in urine and human serum sample.

**2.2. HPLC method.** High-performance liquid chromatography (HPLC) is a powerful tool that enables the separation of complex mixtures into individual components, and is a highly sensitive and reproducible analytical technique. In recent years, HPLC has been combined with many sensitive detection techniques and has experienced continuous improvement of stationary phases, which have improved its sensitivity and specificity. HPLC is currently widely used for the analysis of drugs and dosage forms with respect to quality control, quantitative determination of active ingredients and impurities, monitoring drug blood concentration in patients, and bioequivalence assessment [17,18].

Burdett *et al.* [19] reported the development and validation of a reversed-phase HPLC method combining electrochemical and UV detection after a short gradient run to measure seven purine metabolites (adenosine, guanosine, inosine, guanine, hypoxanthine, xanthine and urate) from the entire purine metabolic pathway. The limit of detection for xanthine using coulometric electrodes was 0.005 mg/L. Using this method, they determined basal levels of these metabolites in mouse brain and serum, as well as in post-mortem human brain. Peak identities were confirmed by enzyme degradation and spike recovery was performed to assess accuracy. The HPLC method provided a sensitive, rapid, reproducible and low-cost method for determining multiple purine metabolites in a single analysis in serum and brain specimens.

Rashed *et al.* [20] developed a rapid liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the analysis of *S*-sulphocysteine, xanthine and hypoxanthine in urine. The analysis was carried out in the negative-ion selected-reaction monitoring mode. The turnaround time for the assay was 7 min. The linear calibration curves for the three biomarkers were obtained in the range of 12–480 μmol/L. This stable isotope dilution LC-MS/MS method was specific, rapid and simple, and provided definitive diagnosis for molybdenum cofactor and isolated sulphite oxidase deficiencies in very small volumes of urine. The method had been used to identify seven new cases of isolated sulphite oxidase deficiency from four Saudi families and one Sudanese family.

**2.3. Electrochemical method.** Since the early 70s electrochemistry has been used as a powerful analytical technique for monitoring electroactive species in living organisms. Generally, the electrochemical detection of xanthine is based on the electrochemical oxidation of the enzymatically generated $H_2O_2$, or the electrochemical reaction of the introduced redox mediators. However, high potentials applied to the working electrode make the biosensor responsive to other electroactive substances such as ascorbic acid and uric acid. In order to eliminate the effect of interferences and to enhance the selectivity of the biosensor, much effort for xanthine detection has been devoted to design the modified electrodes to improve the catalytic properties, sensitivity, and selectivity of electrochemical sensors. Numerous materials, such as metal nanoparticles, polymers, carbon nanotubes, fullerenes, graphenes, and enzymes, have been used as modifiers to construct highly sensitive and selective xanthine biosensors [21-23].

Ibrahim *et al.* [24] prepared a novel electrochemical sensor based on the use of boron doped $CeO_2$ nanocubes modified glassy carbon microspheres paste electrode (B-$CeO_2$NCs/GCPE) and applied it for selective and sensitive determination of xanthine and hypoxanthine individually and simultaneously. From the B-$CeO_2$NCs/GCPE, well oxidation peaks and enhanced peak currents of xanthine and hypoxanthine were observed owing to the excellent catalytic activity of B-$CeO_2$NCs. The practical application of the modified electrode was demonstrated by simultaneously determining the concentrations of xanthine and

hypoxanthine in human biological fluids and in fish meat samples with satisfactory results.

Bas *et al.* [25] presented new xanthine biosensors, XO/Au/PVF/Pt and XO/Pt/PVF/Pt, based on electroless deposition of Au and Pt nanoparticles on polyvinylferrocene(PVF) coated Pt electrode for detection of xanthine. The amperometric responses of the enzyme electrodes were measured at the constant potential, which was due to the electrooxidation of enzymatically produced $H_2O_2$. Compared with XO/PVF/Pt electrode, XO/Au/PVF/Pt and XO/Pt/PVF/Pt exhibited excellent electrocatalytic activity towards the oxidation of the analyte. Under the optimal conditions, the calibration curves of XO/Au/PVF/Pt and XO/Pt/PVF/Pt were obtained over the range of 2.5 $\times 10^{-3}$ to 0.56 mM and 2.0 $\times$ $10^{-3}$ to 0.66 mM, respectively. The detection limits were 7.5 $\times$ $10^{-4}$ mM for XO/Au/PVF/Pt and 6.0 $\times$ $10^{-4}$ mM for XO/Pt/PVF/Pt. Furthermore, the xanthine biosensors achieved satisfied performances in real sample measurements.

**2.4. Capillary electrophoresis method.** In recent decades, capillary electrophoresis (CE) has been developed for trace analysis because of its small sample size of only nanoliters to femtoliters, short analysis time, and biocompatible environments. In addition, rapid separations are feasible with CE because high voltages can be applied to short capillaries and separation efficiency is not dependent on column length. To identify biological and pharmaceutical analysis, CE is coupled to a variety of detectors, including fluorescence, mass spectrometry, and electrochemical detection [26,27].

Mu *et al.* [28] developed a simple CE method with UV detection for the determination of four purines including adenine, guanine, hypoxanthine and xanthine in soybean milk. Acid hydrolysis was used as sample preparation approach, and the effects of several important factors were investigated. Separation of the four purines was achieved in 5 min using 10 mmol $L^{-1}$ sodium borate and a voltage of 25 kV. Under the optimal conditions, the linear range was from 0.5 to 100 mg $mL^{-1}$ and the detection limits were from 0.05 to 0.09 mg $mL^{-1}$. The developed method showed good repeatability for the quantification of the four investigated purines with intra- and inter-day RSD of less than 3.0% and 4.5%, respectively. The optimized acid hydrolysis followed by CE method was successfully applied to determine the purines in different kinds of soybean milk samples.

Causse *et al.* [29] proposed a new fast CE (CE/UV) method for the simultaneous determination of allantoin, uric acid, hypoxanthine and xanthine in human plasma. These products were quantified in the plasma of patients with chronic renal failure before hemodialysis, patients with chronic heart failure and controls. The metabolites were detectable at concentrations of 0.3–0.6 mmol/L. The proposed CE-UV method appeared to be a useful tool for studying physiological and pathological changes of allantoin, uric acid, hypoxanthine and xanthine levels in plasma samples.

**2.5. Other methods.** In addition to these main approaches mentioned above for xanthine detection, still a few special techniques with high sensitivity have been applied. Khajehsharifi *et al.* [30] developed a spectrophotometric method for simultaneous determination of xanthine, hypoxanthine and uric acid in real matrix by orthogonal signal correction-partial least squares. Mateo *et al.* [31] reported the development of a highly sensitive fluorescent multienzymatic biosensor for quantitative xanthine detection. Anik *et al.* [32] designed a centri-voltammetry method that combines centrifugation and voltammetry for xanthine detection.

## 3. Conclusions

Xanthine is a degradation product of purine derivatives. It can penetrate cell membranes and accumulate in extracellular fluids. The concentration level of xanthine in body fluids is a marker of many clinical conditions. Furthermore, xanthine has also attracted much attention in evaluating the meat freshness. Thus, xanthine determination is of clinical and industrial importance [33,34]. This review has highlighted the significant developments in rapid and alternative techniques for the detection of xanthine in recent years. We believe the development of xanthine sensors with better sensitivity and specificity, lower cost, simplicity, along with in vivo analytical technique is still the future effort.

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