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STUDY ON DETECTION METHODS FOR URIC ACID IN BIOLOGICAL SAMPLES

Xiaodong Dong

College of Medicine, Hebei University, Baoding - 071000, China.

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Correspondence to Author:
Dr. Xiaodong Dong

Associate Professor
College of Medicine, Hebei University, Baoding - 071000, China.

Email: xddong@hbu.edu.cn

ABSTRACT: Uric acid is the end-product of purine metabolism and a major antioxidant in humans. Most of the uric acid produced from the catabolism is readsorbed into the blood circulation system after primary filtration and partial secretion by the kidney. Uric acid levels in physiological fluids such as plasma and urine serve as valuable indicators for certain clinical conditions. An elevated uric acid in urine or serum can affect renal function and blood pressure, which is an indicator of gout, cardiovascular and renal diseases, hypertension, etc. Low uric acid levels may be associated with molybdenum deficiency, copper toxicity and worsening of multiple sclerosis. Therefore, detection of uric acid level dissolved in human physiological fluids is indispensable for diagnosis of patients suffering from these disorders associated with altered purine biosynthesis and catabolism. In this article the studies of detection methods for uric acid in biological samples in recent years are reviewed.

INTRODUCTION: Uric acid (UA) [7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione] is a main antioxidant as well as the final product of catabolization of the purine nucleosides, adenosine and guanosine. UA is considered to be an important biomarker in urine and serum, and abnormal concentrations of UA in blood and urine are associated with gout, hypertension, cardiovascular diseases, renal disease and Lesch–Nyhan syndrome, which is related to poor solubility of UA in water, strong interaction between UA and protein molecules and complex redox-dependent pathways *in vivo* [1-3]. Recent studies have also indicated that serum UA is a strong and independent risk factor for type 2 diabetes.

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Thus, it is essential to establish a simple and effective analytical method for the sensitive and selective determination of UA in biofluids for health assessment and disease diagnosis. To date, various analytical methods for the determination of UA in biological samples have been proposed [4, 5]. In this paper, the attributes of different analytical technique for the determination of UA in biological samples in recent years are reviewed.

2. Analytical Methods:

2.1. Chemiluminescence method:

Chemiluminescence (CL) is a well-known and popular analytical method because of its high sensitivity, low detection limit, wide linear working range, and its rapidity, as well as the fact that it can be performed with relatively simple and inexpensive instrumentation, as an excitation source and optical filters are not needed. It has been widely applied in various fields, including clinical diagnosis, biotechnology, pharmacology, food safety, and environmental chemistry [6].

Amjadi *et al.* [7] reported on the CL of graphene quantum dots (GQDs) induced by direct chemical oxidation. It was found that Ce(IV) could oxidize GQDs to produce a relatively intense CL emission. It was attributed to the radiative recombination of oxidant-injected holes and thermally excited electrons in the GQDs. In order to show the analytical application potential of GQDs-Ce(IV) CL system, it was applied to the determination of UA. Under the optimized conditions, the proposed CL system exhibited excellent analytical performance for determination of UA in the range of 1.0×10^{-6} M - 5.0×10^{-4} M with a limit of detection of 5.0×10^{-7} M. The method was applied to the determination of UA in human plasma and urine samples with satisfactory results.

Ma *et al.* [8] developed a simple and sensitive method based on flow-injection CL analysis coupled with luminol-Ag(III) complex for the determination of UA in human urine. The method was based on the CL reaction of luminol with Ag(III) in alkaline solution. UA in the urine could dramatically enhance CL intensities. In optimum conditions, the relative CL intensity had a linear relationship with UA concentration in the range of 1.0×10^{-8} to 5.0×10^{-6} mol L^{-1} with a detection limit of 2.0×10^{-9} mol L^{-1}. The relative standard deviation was 0.71% for 1.0×10^{-7} mol L^{-1} UA.

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 [9-11].

Zuo *et al.* [12] developed an environmentally friendly reversed-phase HPLC method for simultaneous determination of creatinine and UA in human urine samples, which were pretreated by dilution, protein precipitation, centrifugation and filtration, followed by HPLC separations using a reversed-phase C18 column with an aqueous mobile phase of phosphate buffer. This developed method provided a simple, rapid separation and sensitive detection for the species of interest in 10 min with UV detection at 205 nm. Quantitation was carried out by relating the peak areas of the identified compounds to that of hypoxanthine as an internal standard. The detection limits for creatinine and UA were 0.045 and 0.062 mg mL^{-1} respectively. This method has been successfully applied to estimating of creatinine and UA in human urine.

Luo *et al.* [13] developed and validated a liquid chromatography-tandem mass spectrometry method for the determination of UA in human plasma. Separation was achieved on a C18 column by the mobile phase of 30% water and 70% methanol. The calibration curve was established over the range of 0.4096-100 mg/L, and the correlation coefficient was better than 0.99. The intra-day and inter-day relative standard deviations were less than 5.1%. The accuracy determined at three concentrations ranged between 92.7 and 102.3%. This method was successfully applied to an efficacy study of intravenous recombinant urate oxidase produced by *Escherichia coli* in a clinical phase.

2.3. 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 neurotransmitters, CE is coupled to a variety of detectors, including fluorescence, mass spectrometry, and electrochemical detection [14-16].

Xing *et al.* [17] applied a simple and reliable method based on capillary electrophoresis with electrochemical detection to study the effect of aerobic exercises on creatinine and UA concertration in saliva and urine. The detection limits for creatinine and UA were 3.6 µmol L^{-1} and 0.86 µmol L^{-1}, respectively.

This method was successfully used in the rapid analysis of creatinine and UA in saliva samples. After aerobic exercises, creatinine concentration decreased, and UA concentration increased in saliva. In urine, the concentrations of creatinine and UA both increased after exercise.

Zhao *et al.* [18] developed a simple and sensitive method based on CE with CL detection for the determination of UA. The sensitive detection was based on the enhancement effect of UA on the CL reaction between luminol and potassium ferricyanide in alkaline solution. A laboratory-built reaction flow cell and a photon counter were deployed for the CL detection. Experimental conditions for CL detection were studied in detail to achieve a maximum assay sensitivity. The proposed CE–CL assay showed good repeatability and a detection limit of 3.5×10^{-7} M UA. A linear calibration curve ranging from 6.0×10^{-7} to 3.0×10^{-5} M UA was obtained. The method was evaluated by quantifying UA in human urine and serum samples with satisfactory assay results.

2.4. Fluorescence method: In recent years, fluorescence measurements have received more attention owing to their operational simplicity, high sensitivity, good reproducibility and real-time detection. A series of fluorescence probes have been designed for the detection of biomolecules and metal ions. For example, gold nanoclusters (AuNCs), which exhibit molecule-like properties including discrete electronic states and size-dependent luminescence have received great attention. Fluorescent silicon nanoparticles (SiNPs), which have a zero-dimensional silicon-based nanostructure, have been widely used in biology, owing to their good biocompatibility, low cytotoxicity, and antiphotobleaching capability. Colloidal quantum dots (QDs) which exhibit broad absorption profiles and narrow emission with high quantum yields and allow the chemical modification of functional groups on their surfaces make QDs naturally suitable for serving as fluorescent platforms for sensing and imaging in biology [19, 20].

Xu *et al.* [21] developed a highly sensitive and selective sensor for the detection of UA. Gold nanoclusters were synthesized with bovine serum albumin as the template material. Under the catalysis of urate oxidase, hydrogen peroxide was generated, which quenched the fluorescence from the gold nanoclusters. Furthermore, excessive iodide was found capable of enhancing this quenching effect, which significantly improved the sensitivity of the detection. Under an optimized condition, the extent of quenching was found linearly related to UA concentration in the range of 0.7–80μM, and UA as low as 120 nM could be detected. With simple dilutions, blood samples could be analyzed, and satisfactory recoveries were obtained.

Jin *et al.* [22] developed a convenient enzymatic optical method for UA detection based on the fluorescence quenching of ligand-capped CdTe nanoparticles by H_2O_2 which was generated from the enzymatic reaction of UA. The interactions between the CdTe nanoparticles capped with different ligands and H_2O_2 were investigated. The fluorescence quenching studies of GSH-capped CdTe nanoparticles demonstrated an excellent sensitivity to H_2O_2. The detection limit of UA was found to be 0.10 μM and the linear range was 0.22-6 μM under the optimized experimental conditions. These results typified that CdTe nanoparticles could be used as a fluorescent probe for UA detection.

2.5. Electrochemical method: Since the early 70s electrochemistry has been used as a powerful analytical technique for monitoring electroactive species in living organisms. Since UA is an electroactive compound, it can be determined by electrochemical methods. However, it is well known that UA is found in biological fluids with ascorbic acid (AA) and dopamine (DA) and these substances show similar electrochemical behaviors at bare electrodes and interfere each other. Therefore, AA and DA have to be taken into account in the case of electrochemical determination of UA in physiological fluids. In this context, modified electrodes by some materials including biomolecule materials, carbon materials, polymers, organic matters and multi-walled carbon nanotubes have been achieved and their electrochemical performances were tested in the determination of UA [23, 24].

Huang *et al.* [25] determined the UA in human urine by eliminating ascorbic acid interference on

copper(II)-polydopamine immobilized electrode surface. The polydopamine (PD) coating was spontaneously formed through simple immersion of glassy carbon electrode (GCE) in a dilute aqueous solution of DA at acidic solution in the presence of Cu^{2+}. Copper(II) ions were anchored on the surface of GCE to prepare a PD-Cu(II) modified electrode. This modified electrode showed the extraordinary selectivity for electrochemical determination of UA without interference of high concentration of AA. Under coexistence of 5 mM AA, the differential pulse voltammetry peak current of UA gave linear response over the range of 0.06–1.68 mM with detection limit of 24.6 µM. Furthermore, the proposed sensor was ideal for the analysis of UA in human urine samples.

Gupta *et al.* [26] reported simultaneous determination of AA and UA by GCE modified with a nanocomposite film of gold nanoparticles inclusively adhered to MCM-41 type mesoporous silica spheres (Au-MSS). The Au-MSS modified GCE exhibited stable redox peaks. Furthermore, GCE/Au-MSS showed effective electrocatalytic oxidation of both AA and UA diminishing the overpotentials by 580 and 405 mV respectively. Similarly, GCE/Au-MSS diminished the oxidation overpotentials of AA and UA by 200 and 210 mV respectively. Differential pulse voltammetry technique was employed for the simultaneous determination of AA and UA. The limit of detection was found to be 1.65 µM and 2.14 µM for AA and UA respectively. High sensitivity was displayed by the modified electrode and had been successfully employed for the analysis of UA in human blood serum.

2.6. Other methods: In addition to these main approaches mentioned above for UA detection, still a few special techniques with high sensitivity have been applied. Khajehsharifi *et al.* [27] developed an orthogonal signal correction-partial least squares method for the simultaneous spectrophotometric determination of orotic acid, creatinine, and UA in spiked real samples. Chu *et al.* [28] developed an electrochemiluminescent biosensor based on polypyrrole immobilized uricase for ultrasensitive UA detection. Jie *et al.* [29] developed a kinetic determination of UA in human serum by using the uncatalyzed BZ reaction in non-equilibrium steady state.

Amjadi *et al.* [30] developed a silver nanoparticles plasmon resonance-based method for the determination of UA in human plasma and urine samples.

CONCLUSION: Growing demand for accuracy and rapid determination of analytes in human physiological fluids has always been observed. The concentrations of UA in plasma and urine are usually used as valuable indicator for health. Therefore, for the diagnosis of patients suffering from a range of disorder associated with altered purine metabolism, a simple, sensitive, and rapid analytical method for the quantitative analysis of UA is essential for clinical and biomedical fields [31,32]. This review has highlighted the significant developments in rapid and alternative techniques for the detection of UA in recent years. We believe the development of UA sensors with better sensitivity and specificity, lower cost, simplicity, along with in vivo analytical technique is still the future effort.

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