Application of Electrochemical Sensor Based on the Reaction of Potassium Ferricyanide and Uric Acid in Uric Acid Detection

Haowei Wang
Hunan University of Technology, Zhuzhou 412000, China.

Abstract: At present, the number of patients with hyperuricemia and gout in China is increasing year by year, and the demand for uric acid detection is increasing. In the current situation, a uric acid detection chip was designed based on the redox reaction between potassium ferricyanide and uric acid and the principle of electrochemical sensor, and the uric acid content was measured by the electrochemical detection chip and the software PSTrace5.8, and the error of the result was within ±10%. The method is low cost and high accuracy, which can provide a new technical means for uric acid detection.
Keywords: Electrochemical Sensor; Uric Acid Detection; Potassium Ferricyanide

1. Introduction Introduction

1.1 Current situation of hyperuricemia and gout in China

Uric acid (2,4,6 trihydroxypurine, uric acid. UA) is the end product of purine metabolism in the human body. Hyperuricemia is a metabolic abnormality syndrome caused by the disorder of purine metabolism, and it is called hyperuricemia when the blood uric acid level exceeds 420μmol/L on two different days in both men and women. Blood uric acid exceeding its saturation level in blood or tissue fluid can form and deposit sodium urate crystals locally in the joints, inducing local inflammatory reactions and tissue destruction, known as gout; it can be deposited in the kidneys, causing acute nephropathy, chronic interstitial nephritis or kidney stones, known as uric acid nephropathy. There is much evidence that hyperuricemia and gout are independent risk factors for chronic kidney disease, hypertension, cardiovascular disease and diabetes, and are independent predictors of premature death. Hyperuricemia and gout are systemic diseases with multisystemic involvement and have received a lot of attention from multiple disciplines, and their management also requires multidisciplinary involvement. According to relevant data, the prevalence of hyperuricemia ranges from 2.6% to 36% in different races, and the prevalence of gout ranges from 0.03% to 15.3%, with a significant increase and youthful trend in recent years. It has become another common metabolic disease after diabetes mellitus. According to the related survey, by 2022, the number of diagnosed hyperuricemia in China will be as high as 170 million, and the diagnosis rate will be more than 13.3% in some areas with heavy diets, so it is important to develop an accurate method to measure uric acid content for the health of the nation.

2. Main methods of measuring uric acid

Current methods commonly used for uric acid testing include phosphotungstic acid reduction, high performance liquid chromatography, spectrophotometric, enzymatic and uric acid sensor assays and microfluidic paper chip methods. Phosphotungstic acid reduction method is more mature, but it has the disadvantages of poor linearity, precipitation of uric acid and proteins, and susceptibility to interference by reducing substances: high performance liquid chromatography method has high precision, fast analysis, and low sample usage, but it has the disadvantages of pre-treatment of samples, finding suitable chromatographic conditions, and dependence on high precision instruments: spectrophotometric method is

easy and rapid to operate. However, the sensitivity is poor and it is difficult to measure the uric acid content in plasma directly: the advantages of enzymatic method are high specificity and mild action conditions, but its disadvantages of not easy to preserve and high acquisition cost also limit the application of the method; and the microfluidic paper chip method has good development prospects, has the advantages of convenient, fast, green and cheap, but at this stage of development technology is still immature. The uric acid detector method has become a popular method in the field of uric acid detection due to its high sensitivity, good selectivity and practicality. Therefore, this study is based on the uric acid sensing detector method, and the current generated by the redox reaction between potassium ferricyanide and uric acid is measured by an electrochemical detection chip, so that the magnitude of uric acid concentration in the test sample can be visually reflected by the current data.

2. Materials and Methods

2.1 Experimental principle

In this study, a uric acid detection chip was designed based on the redox reaction between potassium ferricyanide and uric acid, with the principle of (1) $K_3 Fe(CN)_6$ + uric acid → $K_4 Fe(CN)_6$ + uric acid oxide.

(2)$K_4 Fe(CN)_6 - e^- \rightarrow K_3 Fe(CN)_6$ Uric acid is oxidized by potassium ferricyanide, which loses electrons to generate potassium ferricyanide, and then the oxidation potential is applied to the surface of the working electrode, and potassium ferricyanide loses electrons to become potassium ferricyanide again, and the oxidation current on the surface of the uric acid detection chip is collected by the detection instrument to realize the conversion of the uric acid concentration size of the detected sample into the value of the circuit. The uric acid concentration in the sample can be measured by the detection circuit. The instrument is designed based on the principle of electrochemical sensor, and the oxidation potential is applied to the surface of the working electrode of the uric acid detection chip relative to the reference electrode, and the oxidation current generated on the surface of the working electrode of the cell phone due to the oxidation of uric acid is converted between the chemical reaction signal and the electrical signal by the electrochemical detection chip to obtain more accurate results of uric acid.

2.2 Experimental materials and apparatus

Experimental material: uric acid quality control solution with different uric acid concentrations (Since human blood samples are not easily available and inconvenient to use, a uric acid quality control solution was designed to replace the blood in this experiment, as follows: 100 mL of ultrapure water was taken, to which the following substances were added in order and the mass concentrations of each substance were made: sodium chloride 0.58% (w/w), phosphate buffer 1.82% (w/w) (pH 7.4), polyethylene glycol (molecular weight 40,000) 4 g/L, cuscuta red 0.1% (w/w), ProClin 300 0.04% (w/w). Uric acid was added to the above base solution at different concentrations (Uric acid was pre-dissolved with 0.lmol/L NaOH) to prepare a concentration gradient of 0.25 mmol/L, 0.5 mmol/L, 0.8 mmol/L, and 1.0 mmol/L of Uric acid quality control solution, respectively.)

Uric acid detection chip (by screen printing a conductive silver film on a PET sheet as a silver conductive layer connecting the three electrodes, and a carbon film printed on the front of the silver conductive layer as a counter electrode, working electrode, reference electrode, and suction sample judgment electrode. The reference electrode and the counter electrode share a common electrode, and the working electrode and the reference electrode form a circuit to measure the potential value of the research electrode. The working electrode and the counter electrode form another circuit for measuring the current value. The aspiration judgment electrode is used to detect whether the sample fills the entire biochemical reaction zone. Modify the reaction active layer on the electrode surface: the reaction layer solution is configured with ascorbate oxidase, potassium ferricyanide, carboxymethyl cellulose and phosphate in certain ratio, where ascorbate oxidase is the anti-interference substance, potassium ferricyanide is the electron mediator for the reaction with uric acid, and

carboxymethyl cellulose is the film-forming auxiliary component. 1.5 μL of the configured solution is sucked and dropped onto the reaction working area of the prepared electrode substrate, and (Dried at 37℃ for 20 min, and after drying, double-sided adhesive and hydrophilic film were applied to produce the uric acid detection chip.)

Test apparatus: open electrochemical detection chip, computer with detection software PSTrace 5.8

2.3 Experimental step design

Step 1: Insert the uric acid test chip into the electrochemical test chip and connect the electrochemical test chip to the computer via the data cable.

Step 2: Open the software and apply a drop of uric acid quality control solution to the surface of the uric acid test chip after the current display is smooth.

Step 3: Read the software value after 15s and compare it with the actual value.

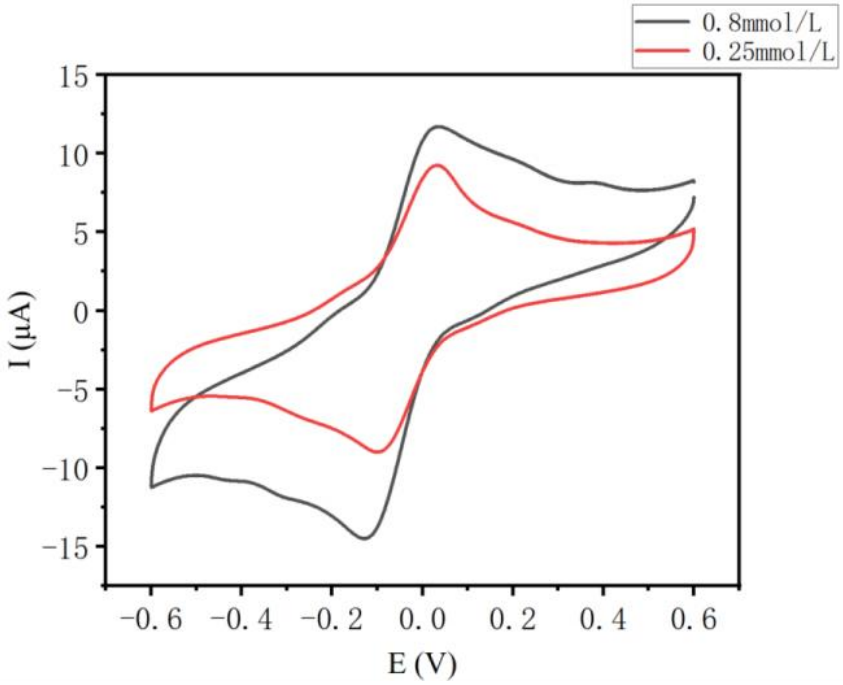
Step 4: Repeat the above steps using different concentrations of uric acid quality control solution.

2.4 Analysis of experimental results

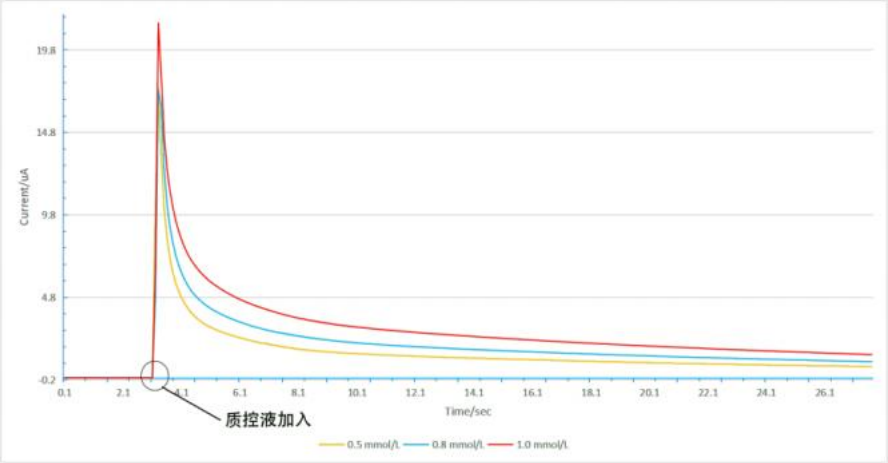
After several tests, it was shown that the measurement error was within ±10%, and the uric acid detection chip designed by the modified method has a high accuracy for measuring uric acid concentration.

3. Discussion of experimental results

At the end of the experiment, for further determination of the accuracy of the results, i.e. further testing of the functionality of this uric acid detection chip for uric acid sensing recognition, cyclic voltammetry and constant potential methods were used and tested again, as recorded below.



Cyclic voltammograms of uric acid quality control solution at different concentrations. It can be seen from the figure that a pair of redox peaks appear between -0.2 V and 0.2 V during cyclic voltammetry scan, which is the redox of Fe^{3+} /Fe^{2+} ion pair with the oxidation peak potential at about 0.03 V. As the concentration of uric acid QC solution increased from 0.25 mmol/L to 0.8 mmol/L, the redox peak current in cyclic voltammetry scan increased, which demonstrated the good sensing function of the chip for uric acid.



Relationship between uric acid quality control solution and response current for different uric acid concentrations at constant potential.

It can be seen from the figure that at a constant potential of 0.12 V, when the uric acid concentration in the uric acid quality control solution sample increased from 0.5 mmol/L to 0.8 mmol/L and then to 1.0 mmol/L, the response current magnitude increased sequentially with the increase of uric acid concentration after the addition of uric acid quality control solution, which further proved that the detection chip has a good function of uric acid sensing and recognition.

These two measurements further validate the accuracy of the results of the uric acid test chip.

4. Conclusion

The experiment showed that the electrochemical sensor based on the reaction between potassium ferricyanide and uric acid is more accurate in uric acid detection. Although the human blood experiment has not been conducted due to technical reasons, this experiment still provides a new idea for the design of uric acid detection chip, and we hope to provide our share for better uric acid detection.

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