Application of Chitosan-based Biosensor for Uric Acid Detection

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Abstract

An amperometric non-enzymatic sensor for uric acid, based on a chitosan-graphene modified screen-printed electrode was developed. In this sensor, the graphene was electro reduced by the one-step constant potential method, and chitosan was deposited at the same time to obtain the screen-printing electrode modified by chitosan and graphene. It was found that the electrode had a good linear relationship with uric acid in the range of 7×10^{-5}M to 8×10^{-4}M. The linear equation was $I (\mu A) = 0.011c (\mu M) - 0.24$, and the correlation coefficient was R=0.9995. In addition, the uric acid sensor has good anti-interference, high test accuracy, and good reproducibility, which will be used for actual sample testing and the portable equipment have a good commercial development prospect.

Keywords

Screen-printed electrode; Uric acid; Enzyme-free detection; Portable equipment

Introduction

Uric acid（UA）is the final product of purine metabolism, and timely and rapid measurement of uric acid concentration in body fluid is of great value in the diagnosis and monitoring of related diseases(Fu, Wan & Tu, 2009) (Lu, Du, Xue, Bai, Yin, Li, Zhang & Yang, 2007). UA exists in blood and tissue fluid, and it mainly exists in the form of sodium urate at pH 7.4. Uric acid is continuously produced and excreted in the human body. The normal uric acid level of healthy adults is 250-750 mg/L in urine (Lu, Du, Xue, Bai, Yin, Li, Zhang & Yang, 2007) (Wang, Zhang, Chen, Zhu, Xian & Jin, 2003). However, when eating a lot of high-nucleic acid foods or due to diseases, the source of uric acid in the blood increases, and the excretion of uric acid increases with urine. It has been shown that abnormal UA concentration may cause the following diseases: gout, hyperuricemia, and Lesch-Nyhan syndrome. In addition, UA as a reducing agent can consume oxygen free radicals and prevent them from damaging tissues and cells. Abnormal uric acid in the human body is a response to human diseases. Therefore, it is of great significance to develop a simple, accurate, and feasible instrument to detect uric acid

content. It can understand whether the cell degradation rate in the body is too fast or the body's ability to excrete uric acid. If the uric acid concentration is controlled within the normal physiological range, the uric acid syndrome can be controlled. (Lu, Du, Xue, Bai, Yin, Li, Zhang & Yang, 2007)

My goals for this paper are as follows，1) Whether the electrochemically reduced graphene-chitosan modified screen printing electrode can be modified on the electrode by electrochemical method, and what is the most suitable deposition condition? 2) Whether the modified electrode obtained can be corresponding to UA; 3) The corresponding linear range, stability, reproducibility, and anti-interference of the modified electrode for UA; 4) Whether the transformation from the research stage to industrialization is feasible; 5) Portable equipment stability, operability, etc.

This paper is divided into the following parts: Literature Review, Experiment, Results and Discussion, Conclusion, Evaluation, and Resource Assessment.

In the literature review section, I will introduce the principle of electrochemical detection of uric acid, the principle of an electrochemical sensor, the introduction of electrode, the preparation method of chitosan modified electrode, the introduction of preparation method of graphene, screen printing technology, and experimental hypothesis.

In the experimental part, the preparation method of chitosan-electrochemical reduction graphene and the preparation process of the modified electrodes are described in detail. In the results part, the electrode is illustrated with diagrams and the linear response of the electrode to uric acid is analyzed. Finally, I discussed the data I

obtained and reached conclusions on-chip stability, reproducibility, selectivity, and anti-interference, and at the same time summarized the current electrochemical detection problems. In the self-assessment part, I summarized my own strengths and weaknesses. I have the ability to discover and solve problems, but at the same time, I also have the shortcomings of limited knowledge. My biggest gain is being able to apply what I have learned, combining theory with practice, and improving by discovering problems, solving problems, and searching for documents.

I will track and verify each cited document in the resource evaluation section, and explain whether each cited document is authoritative and reliable.

Literature Review

Principle of Electrochemical Detection of UA

UA is an electrochemically active substance, which can be oxidized under appropriate conditions. However, the oxidation situation is different under different conditions. Some scholars have studied the oxidation situation of UA at pH=1.5~9.5. The oxidation process of UA is shown in Figure 1: First, it loses two e- and two H+ and then forms an unstable anionic quinoid material. When the pH value is greater than or equal to 6, the unstable anionic quinoid material will show the affinity for water molecules, which will eventually form stable allantoin after decomposition.
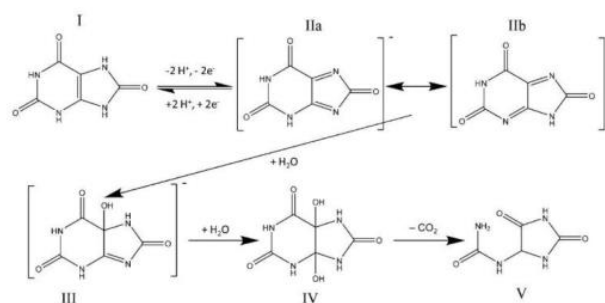
Figure 1 The electrochemical oxidation process of UA

Figure 2 electrochemical sensor devices principle

The currently reported methods for detecting uric acid mainly include the enzyme analysis method (Markas & John, 1994), voltammetry method (Ren & Ma, 1997), HPLC method (Marion, 1994), and so on. Enzymatic analysis and high-performance liquid-phase methods have high sensitivity, but they require expensive reagents and instruments and require trained professionals, so they are not popular. Voltammetry uses electrochemical methods to detect substances. The unique program design can realize a portable testing instrument like a blood glucose meter, realizing the industrialization of the research direction. Compared with the above traditional methods, voltammetry technology has the following advantages: high selectivity, low cost, and low time consumption.

The Principle of Electrochemical Sensor
An electrochemical sensor is a type of device that can sense (respond to) biological or chemical quantities and uses electrochemical principles to convert the concentration change of the measured component into an electrical signal and output it. Its main components include signal recognition components, signal processing and conversion components (electrodes), and signal output components. The working principle is shown in Figure 2 below:
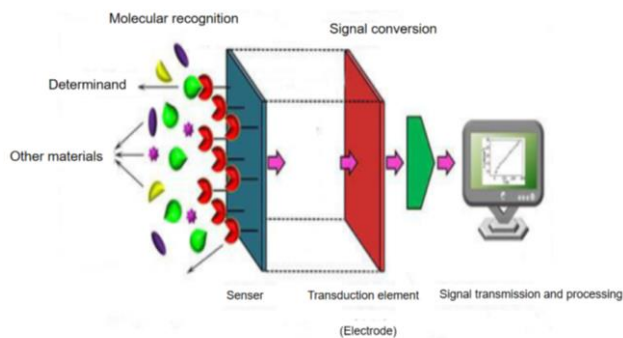

The detection signal of the electrochemical sensor can be divided into points. Current and conductance, and electrochemical sensors can also be divided into three categories according to the detection signal. A potential sensor refers to the sensor based on the relationship between the electrode potential and the ion activity of the measured component, and the change of the electrode potential infers the change of the solution ion concentration. The representative potentiometric sensor is also a pH sensor. Current sensor: The principle is to set the electrode and the solution interface to a constant potential so that the measured substance changes in the potential to generate a current. When the diffusion control conditions are met, the limiting current has a linear relationship with the concentration. A conductivity sensor refers to a sensor that undergoes an oxidation or reduction reaction of the measured substance, which causes a change in the conductance in the electrolyte solution, and detects the change in conductance. Amperometric electrochemical sensors are the most widely used of the three electrochemical sensors. It provides a highly sensitive method for detecting micro-scale electroactive substances.

This research is current sensor research, the methods used mainly include chronoamperometry, cyclic voltammetry, differential pulse voltammetry, and other common methods. Generally, cyclic voltammetry is mainly used to judge the microscopic reaction process of the electrode surface, the reversibility of the electrode reaction, and the "touch condition" of the inorganic preparation reaction. Due to its high sensitivity, differential pulse voltammetry is generally used to investigate the linearity of the electrode to the substance. Chronoamperometry is generally

used for the reaction mechanism of organic electrochemistry and the electrochemical deposition of substances.

Introduction of Electrode

The Electrode

The electrode is the core of the electrochemical sensor and is the place where the determinant has an oxidation or reduction reaction. The performance of the electrode directly determines the performance of the electrochemical sensor. According to different functions and purposes, electrodes can be divided into working electrodes, reference electrodes, and counter electrodes. The working electrode is the place where the research object reacts, so the working electrode is also called the research electrode. We already know that the reference electrode potential is constant, and the purpose is to make the potential changes in the battery come from the working electrode. The function of the counter electrode is only to form a loop with the working electrode to realize the polarization of the working electrode, so it is also called the auxiliary electrode. The working electrode and the counter electrode can form a two-electrode detection system, and the working electrode, the reference electrode, and the counter electrode can be combined into a three-electrode detection system.

It is particularly pointed out that the three-electrode system is divided into two circuits according to different functions, namely a polarization circuit (composed of a working electrode and counter electrode) and a potential measurement circuit (composed of a working electrode and reference electrode). The polarization loop is the loop where people study the polarization current, and the potential measurement loop is used to study the potential change between the working electrode and the reference electrode. Among them, the potential measurement circuit has a very high input impedance and almost has no current flows. It can be seen that a three-electrode system with two different functional circuits can study current and potential at the same time to obtain the polarization curve of a single electrode (working electrode). Therefore, the current three-electrode detection system is the mainstream system in electrochemical detection.

Electrodes can be divided into metal and non-metal electrodes according to their materials. Non-metal electrodes mainly include carbon electrodes and paper electrodes; metal electrodes mainly include platinum, gold, and silver electrodes. Different electrochemical sensors are used in different places and require different functions. Some require strong anti-interference, while others require low detection limits and high sensitivity. Generally, chemical or physical modification is required, because bare electrodes without secondary treatment cannot meet multiple requirements at the same time.

Chemically Modified Electrodes

The emergence of chemically modified electrodes (CMEs) has broadened the scope of traditional electrochemical research, opened up the research field of artificially controlling the surface structure of electrodes from the chemical state, and opened up a new way to modify electrodes at the molecular level. In a broad sense, artificial CMEs refer to all electrodes whose surfaces have been molecularly designed and manually tailored. In addition to these two modification methods, any chemical change to the electrode interface to make it show different properties from the material itself is generally called CMEs. The purpose of CMEs is to make the electrodes perform specific functions by performing certain specific chemical modifications on the electrodes so that certain

reactions can be selectively performed on the electrodes. Compared with other electrode concepts, a significant feature of CMEs is that the surface of the electrode is covered with a thin film with different chemical groups that can meet the requirements by spin coating and other methods according to different needs. The film size ranges from a single molecule to a few microns.

According to the scale of the microstructure on CMEs, chemically modified electrodes can be divided into a single molecular layer, a multi-molecular layer (mainly polymer film), and their combination. The modification method of the electrode surface varies according to the electrode base material, function, and type. Among the common methods for preparing monolayers are covalent bonding, adsorption, and constant potential deposition. The covalent bonding method differs depending on the electrode substrate and the bonding method. When the substrate is carbon-based, bonding grouping is used to achieve covalent bonding; When the electrode substrate is metal or semiconductor-based, silanization can be used to achieve covalent bonding. Both bonding methods are realized through organic synthesis on the surface. The methods for preparing multi-molecular layers are mainly the polymer film method and the vapor deposition method. The polymer film method can be divided into monomer film and polymer film according to the properties of the film. The monomer film modification methods include electrochemical polymerization, chemical polymerization, plasma polymerization and radiation polymerization. The polymer film can be modified by dip coating, drip coating, spin coating, and electrodeposition on the surface of the electrode.

In this study, a graphene-chitosan modified screen-printed microelectrode was used to achieve portable detection of uric acid in urine.

Preparation Method of Chitosan Modified Electrode
Chitosan is a product of chitin deacetylation. Its structure is shown in Figure 3. It has the advantages of non-toxicity, good biocompatibility, biodegradability, low price, and good chemical modification ability (Kumar, 2000). Because chitosan is a natural polymeric cation with a natural polymer network structure, it can effectively maintain the biological activity of biomolecules without leaking, has good adsorption and stability after film formation, and has been widely used immobilization of biomolecules and preparation of modified electrodes (Zhang, Mullens & Gorski, 2007)(Veerapur, Gudasi, & Aminabhavi, 2007), such as the determination of metal ions(Gao, Lee, Oshima & Motpmizu, 2000), non-metal ions (Zeng, Zhu, Wang & Lu, 2005), and organic molecules (Kumar, 2000). This broadens the application of chitosan in sensors. Since the biosensor prepared by chitosan has the advantages of low cost, fast response time, and high sensitivity, and chitosan can maintain the stability of the sensor, the research on chitosan biosensor has attracted more and more attention. The preparation methods of chitosan biosensors mainly include the covalent bonding method, an adsorption method, combination method, a coating method, electrodeposition method, self-assembly method, and sol-gel method.
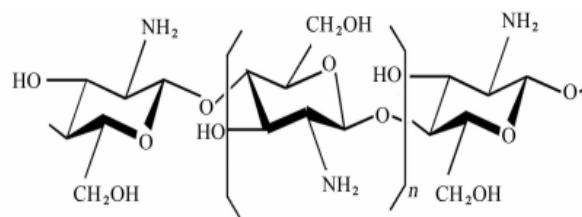

Figure 3 Schematic diagram of chitosan structure

Covalent Binding Method

The covalent bond is legal Covalent bonding method uses a chemical reaction to covalently bond chitosan to the electrode surface, generally in two steps, the first step is to oxidize the electrode surface to introduce bonding groups; the second step is to perform surface organic synthesis. The chitosan is fixed on the electrode surface（Lian, Sun, Xu & Wang，2007）through a covalent binding reaction. Li et al. (Li, Xu & Sun, 2002) modified chitosan on the surface of a glassy carbon electrode by covalent bonding and measured NO^{2-} by anodic stripping voltammetry. The modified electrode has good adsorption and selectivity for NO^{2-} and applied this sensor for the determination of NO^{2-} in rainwater.

Adsorption Method

The adsorption method is to fix chitosan through non-covalent interactions (such as electrostatic attraction, hydrogen bonding, hydrophobic interaction) on the surface of the electrode substrate. Compared with covalently bonded monolayers, the method through non-covalent interaction is simple and low in cost. Feng et al. (Feng, Zhao, Xu & Chen, 2005) adsorbed three types of heme protein, myoglobin, and cytochrome c on a gold electrode, and then fixed it with chitosan. The sensor had a good response to hydrogen peroxide.

Combination Method

The combination method is to simply combine chitosan and electrode material to make a combined modified electrode. For example, a chemically modified carbon paste electrode can mix a certain amount of chitosan with a certain amount of carbon powder and paraffin, then compress it in a glass tube, smooth the electrode, and then draw it out with a wire after washing to make a chitosan sugar electrochemical sensor. Janegitz et al. (Janegitz, Marcolino-Junior, Campana-Filhoc, Fariaa & Fatibello-Filhoa, 2009) used glutaraldehyde and epichlorohydrin as cross-linking agents to prepare cross-linked chitosan and mixed the crosslinked chitosan with functional carbon nanotubes and mineral oil to prepare carbon nanotube paste electrodes. This serves as a sensor for detecting copper ions. The electrochemical behavior of Cu^{2+} on the electrode was characterized by linear scanning anodic stripping voltammetry, and the samples of industrial wastewater, tap water, and human urine were measured by this method.

Coating Method

The coating method is to use an appropriate method to drop chitosan solution on the surface of the electrode substrate to form a thin film. The method is simple to prepare and facilitates the design of multiple functions. Coating methods can be divided into dip coating methods and spreading methods. The dip-coating method is to immerse the electrode in a chitosan solution, then take it out, evaporate the solvent, and form a modified film on the electrode surface. The unfolding method uses a pipette or micro-injector to pipette a certain volume of chitosan solution on the surface of the electrode and spread it horizontally, evaporate the solvent and form a film. Liu et al. (Liu, Honma & Zhou,2005) prepared a mixed solution of tyrosinase-chitosan and applied it to the surface of the glassy carbon electrode substrate to determine the neurotransmitter metabolite triquetra-dihydroxyphenylacetic acid (DOPAC). The sensor has the characteristics of fast response time, high sensitivity, and good selectivity

Electrodeposition Method

The electrodeposition method uses electrochemical means to oxidize or reduce the polymer to a less soluble valence state so that the polymer can be firmly deposited on the electrode surface to form a modified layer. Wang Xueying

et al. (Wang, Deng & Tu, 2008) applied controlled potential electrolysis to electrodeposition of Prussian blue (PB)/chitosan (CTS) modified film on gold electrodes. The results show that PB and CTS are deposited on the electrode at the same time, which is more stable than pure PB modified film, has good electrochemical activity and electrocatalytic performance for hydrogen peroxide, which lays a good foundation for the development of electrochemical biosensors based on enzyme-catalyzed reactions basis. In this study, chitosan and graphene oxide are deposited on the screen printing electrode in one step by electrodeposition. In the presence of chitosan, the electrochemical modification of graphene oxide to the silk is easier. On the screen-printed electrodes, a portable biosensor was prepared.

Self-assembly Method

The self-assembly method is an ordered molecular assembly system formed by the spontaneous adsorption of active molecules on the heterogeneous interface through chemical bonds. It can be designed at the molecular level according to people's expected goals. Constantine et al. (Constantine, Mello, Dupont, Cao, Santos, Oliveira, Strixino, Pereira, Cheng, Defrank & Roger, 2002) used self-assembly technology to assemble chitosan/polythiophene-3-acetic acid (PTAA) on a hydrophilic quartz plate, and then immobilized organophosphorus hydrolase on chitosan/PTAA to make an enzyme sensor. The sensor can interact with toxic organophosphorus pesticide paraoxon to change the spectral characteristics of PTAA, so as to achieve the purpose of detecting paraoxon.

Sol-gel Method

In recent years, organic-inorganic hybrid composite materials based on sol-gel technology have flexibility and ease of modification, as well as the rigidity and stability of inorganic substances, which are more conducive to keeping biological molecules alive. It is used in the development of biosensors. Tan Xuecai et al. (Tan, Huang, Xu, Luo, Zou & Cai, 2004) utilize in-situ sol-gel (Sol-Gel) technology preparation of CTS/SiO2 organic-inorganic hybrid film with chitosan (CTS) and tetraethoxysilane (TEOS) for the immobilization of horseradish peroxidase (HRP), infrared spectroscopy and scanning electron microscopy characterized. The catalytic performance of the enzyme electrode was studied by cyclic voltammetry and chronoamperometry. The results show that when used in the development of immobilized enzyme biosensors, the hybrid membrane not only has a fast response time to the substrate, but also can better maintain the enzyme activity.

Introduction of Preparation Method of Graphene

In 2004, Geim of the University of Manchester in the United States discovered graphene, which has a perfect two-dimensional periodic planar structure, and has the excellent properties of graphite and carbon nanotube materials, such as high thermal conductivity and high mechanical strength, and more prominent point is its unique electronic structure and electrical properties (Net, Guinea, Perez, Novoselov & Game, 2009) (Game & Novoselov, 2007)(Stankovich, Dikin, Dommett, Kohlhaas, Zimney, Eric, Richard, SonBinh & Rodney, 2006). However, traditional preparation methods, especially the reagents and products used in chemical reduction methods, will pollute the environment. (Guo, Wang, Qian, Wang & Xia, 2009)Therefore, there is an urgent need to find a green method for preparing graphene. Recent studies have found that the method of preparing graphene by direct electrochemical reduction of graphene oxide is extremely effective, and electrochemically reduced graphene has been applied in many fields, such as sensors (Guo, Wang, Qian, Wang & Xia, 2009), capacitors(Liu, Ou, Wang, Liu &

Yang, 2011), and fuel cells(Zhou, Chen, Wang, Sheng & Xia, 2010). However, the chemical reagents used in the process of preparing graphene by chemical reduction, such as hydrazine hydrate, are harmful to humans and the environment. However, the electrochemical reduction method can completely avoid the use of harmful reagents and is an environmentally friendly and fast method for preparing graphene.

Screen Printing Technology

Screen printing technology is a traditional and practical technology with a long history. With the development of microelectronics technology, screen printing has become the most commonly used printing method in the application field of electronic products. It is widely used in printed circuit boards, integrated electronic boards, and various kinds of thin-film battery circuits, and other aspects (Wang, 2018). Screen-printed electrodes are one of the more common applications, which have the advantages of low production cost, fast response speed, good reproducibility, less sample usage, and production automation. They have been successfully commercialized. Currently, screen-printed electrodes have been widely used in biosensors.

I use the electrochemical method to reduce graphene and chitosan to the screen-printed electrode in one step. The method is simple and easy to implement, and the reagents used are pollution-free.

Experiment

Test Prediction

In this study, I try to combine the use of graphene and chitosan creatively, the graphene and chitosan are restored to the screen-printed electrode in one step through electrochemical methods, and they are integrated into a portable

household method for detecting the content of uric acid in urine through screen printing and electrochemical technology. The patient can quickly test uric acid at home, reducing the inconvenience of the patient going to the hospital and reducing the patient's medical expenses. The method is simple and easy to implement, and the reagents used are free of pollution.

Test Equipment and Reagents

CHI850D electrochemical workstation, electrochemical deposition equipment (independent development), handheld equipment (independent development), Hitachi S-4800 scanning electron microscope, graphite oxide (modified Hummers method (Chen, Zeng, Liu, Dong, Liu, Wu, Yang & Li,2009) (Hummmers, & Richard, 1958), $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, NaOH, Acetic acid are all analytical pure Sinopharm reagents, chitosan (self-produced), screen-printed electrodes (self-produced).

Solution Preparation

Preparation of Chitosan Graphite Oxide

1)Disperse 20mg of graphite oxide(GO) in 50ml of 0.5% acetic acid solution and sonicate for 5 hours to obtain a uniform solution;

2)Take another 0.5g of chitosan and dissolve it in the mixture in 1), stir it evenly, and adjust the pH of the mixture to 5.0 with 1% sodium hydroxide solution.

Preparation of Phosphate Buffer

Add 0.045g of sodium dihydrogen phosphate dihydrate and 0.563g of disodium hydrogen phosphate dodecahydrate into a 1 L volumetric flask, then add water to make

Preparation of Modified Electrodes

1) Insert the blank screen-printed electrode (a

single piece as shown in Figure 4) into the electric control needle of the electrodeposition device in batches, and put the working electrode end of the screen-printed electrode into the prepared chitosan oxidation graphite solution.

2) After all the devices are installed, use -0.8 V (vs. SCE) constant potential reduction of graphite oxide while electrodepositing chitosan for the 30s to obtain electro-reduced graphene-chitosan modified screen printing electrode, marked as EG/CS/SP electrode.
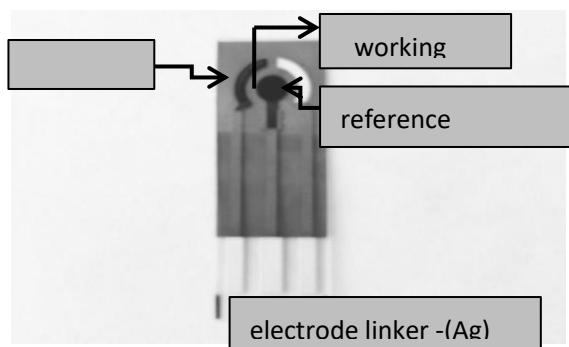

Figure 4 Plan view of a blank screen-printed electrode

Results and Discussion

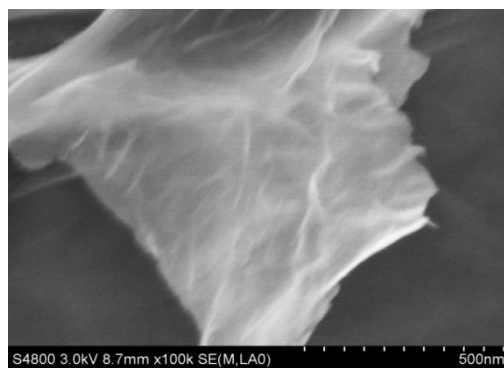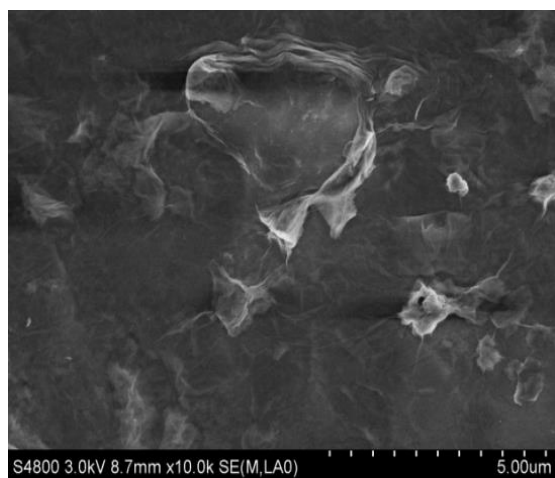### FE-SEM Image of Modified Electrode

Figure 5 FE-SEM Image of EG/CS/SP electrode

It was found that the electrochemically reduced graphene shows a more obvious graphene-specific wrinkle state, and there is no obvious agglomeration.

Electrochemical Catalysis of EG/CS/SP Electrode on Uric Acid
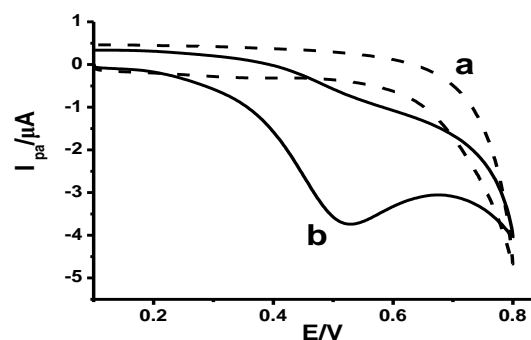

Figure 6 Cyclic voltammetry curve of EG/CS/SP electrode in PBS solution with or without uric acid buffer: a without uric acid; b 2M with uric acid

It can be seen from Figure 6 that there is no redox peak on the EG/CS/SP electrode without uric acid (a), but after the addition of uric acid (b), the EG/CS/SP electrode has significant oxidation at about 0.52V. The peak indicates that the electrode has a good catalytic effect on uric acid, and electrochemical methods can be used to detect uric acid.

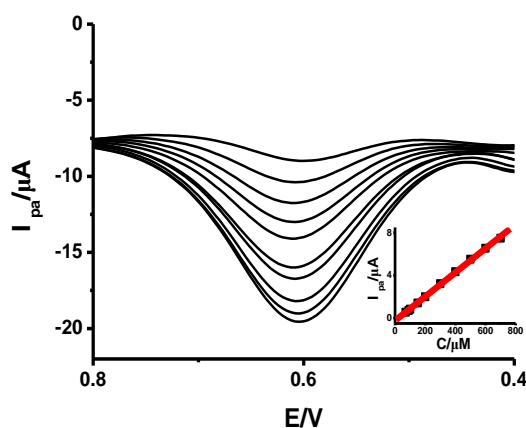**Linear Response of EG/CS/SP Electrode to Uric Acid**

Figure 7 Differential pulse voltammogram of EG/CS/SP electrode catalyzed by different concentrations of uric acid. The inset is the line graph of the electrode catalyzed by uric acid

It can be seen from Figure 7 that the EG/CS/SP electrode has a good linear response to uric acid in the range of 7×10^{-5}M to 8×10^{-4}M. The linear equation is I(μA)=0.011C(μM)-0.24(R= 0.9995).

Sensor Stability and Reproducibility
In order to test the stability and reproducibility of the biosensor, the EG/CS/SP electrode was studied. The same EG/CS/SP electrode was used for 10 parallel tests of 2mM uric acid. The results showed that relative standard of 10 tests deviation is less than 6.5%; 10 identical electrodes prepared in the same batch can detect 2mM uric acid simultaneously, and the relative standard deviation is less than 5.8%; 10 electrodes prepared from different batches are randomly selected to detect 2mM uric acid, and the relative standard deviation is less than 8.2%. The electrode prepared by this method has good reproducibility and stability.

Selectivity and Anti-interference
There are often interferences from other substances in the detection of actual samples.

Some interfering substances that may exist are detected to investigate the selectivity and anti-interference of the modified electrode. The results showed that for 1.0×10^{-6}M cholesterol, 3×10^{-6}M ascorbic acid, 5×10^{-6}M dopamine, etc. produced 0.5%, 0.3% and 1.5% current changes, respectively. These changes are very small compared with the current value of uric acid, so the interference produced is almost negligible. Others such as glucose, urea, lactic acid, cysteine and bovine serum albumin and most of the metal ions (100 mM K^+, Na^+, Mg^{2+}, Ca^{2+}, NO_3^-, Cl^-; 50 mM Cu^{2+}, SO_4^{2-}) don't have interference. It shows that the sensor has good selectivity and anti-interference performance.

Detection of Actual Samples
In order to verify that the EG/CS/SP electrode has practical application value, we used the standard addition method to detect the morning urine of the actual sample with the electrode, and compared the results with the portable device detection and the traditional high-performance liquid chromatography. The results are shown in Table 1. The recovery rate is 98.1%-102.0%, and there is no big difference in the test results between the three methods. It can be seen that the screen-printed electrode prepared by this research has great commercial development space.

Table 1 Comparison table of actual samples tested by different methods

Method	Equipment	Addition (μM)	Detection amount (μM)	Recovery rate (%)	Relative deviation (%)
EG/CS/SP Electrode	CHI850D	0	90	-	-
		80	168.5	98.1	3.1
		160	253.2	102.0	2.7
EG/CS/SP Electrode	Portable device	0	90	-	-
		80	170.2	100.3	3.5
		160	249.5	99.7	3.8
HPLC		0	90	-	-
		80	169.8	99.8	2.5
		160	251.5	100.9	3.0

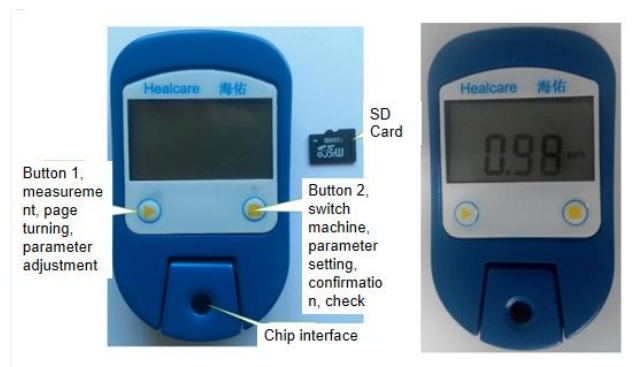Footnote: The portable device is shown in Figure 8 below:

Figure 8 Portable device

The using method is as follows:

1) Turn on the side switch key of the device

2) Turn on the blue cover at the device chip interface, then place the working electrode of the electrode chip facing up and the electrode pins facing inward, at last, insert it into the device and cover it with a blue cover;

3) Add determinant to the working electrode of the electrode chip(namely add determinant to the hole of the electrode chip interface), then press the button and begin to measure. The result will be shown in 1min, which is the actual concentration of the determinant.

Conclusion

This study combines the advantages of screen printing technology and electrochemical technology to ensure the reproducibility and stability of the modified electrode prepared in the same batch and between different batches. The prepared modified electrode can better realise the detection of uric acid samples, especially the self-developed portable device that has good commercial development and clinical application prospects.

The polysaccharide bioelectrode chip belongs to the blue ocean high-tech industry field. Since the birth of the first biochip in 1992, biochip technology has developed rapidly, and the market has expanded year by year. Due to the huge market prospects of biochips, the US government and industry have invested nearly US$2 billion in the research, development, and industrialization of biochips in the past 10 years. At the same time, Europe and Japan have also increased investment in this industry. China also attaches great importance to the biochip industry. Various levels of science and technology and industrial development plans, such as the National Science and Technology Research Plan, the Natural Science Foundation, and the Torch Program, have all listed them as priority development targets.

But after the experiment, I found that the industrialization of the project realization still has a lot of problems. The problems of electrochemical detection are summarized as follows:

1) Product standard: The product prepared is a kind of enzyme-free uric acid sensor, which lacks clear domestic and foreign standards, so it makes the research lack scale.

2) Specificity: Compared with commercially available enzyme test papers, the enzyme-free uric acid sensor prepared by this study lacked specificity when detecting actual samples, for the reason of the complexity of the actual sample composition.

3) Handheld device: Although the handheld device for testing Haiyouxin biochips has been basically formed, there are still many problems in the software, such as: unable to set parameters, no storage function, unable to achieve the purpose of testing, etc.

4)Industrialization transformation: In the transformation of small-scale test results to Chinese-style products, the stability and reproducibility of substrate electrodes prepared by Chinese-style equipment need to be further investigated.

Conflict of Interests: the author has claimed that no conflict of interests exists.

References

1. Fu Zhiqiang, Wan Mingyi & Tu Yifeng, Research on uric acid biosensor based on nano-MnO2 ferrocene modified screen-printed carbon electrode. Chinese Journal of Analytical Science.2009, 25, 79-82.

2. Lu Xuxiao, Du Xia, Xue Ling, Bai Huiping, Yin Xiaoyan, Li Baozhu, Zhang Yaping & Yang Yunhui, Development of a uric acid sensor modified by carbon nanotubes and nano-zinc oxide. Journal of Yunnan University (Natural Science Edition)2007, 29, 216-221.

3. Wang Xiaoli, Zhang Fenfen, Chen Yan, Zhu Ziqiang, Xian Yuezhong & Kingston, Development and application of carbon nanotube modified uric acid sensor. Chemical Sensor.2003, 023, 55-61.

4. Markas A. T. Gilmartin &John P. Hart, Novel, reagentless, amperometric biosensor for uric acid based on a chemically modified screen-printed carbon electrode coated with cellulose acetate and uricase. Analyst 1994, 119, 833-40.

5. Ren Nailin & Ma Rongxuan, Determination of Uric Acid in Plasma and Urine by Adsorption Stripping Voltammetry. Analysis and Testing Technology and Instruments 1997, 3, 142-146.

6. Marion A. Ross, Determination of Ascorbic Acid and Uric Acid in Plasma by High-performance Liquid Chromatography. Journal of chromatography. B, Biomedical applications 1994, 657, 197-200.

7. Majeti N.V. Ravi Kumar, A review of chitin and chitosan applications. Reactive and Functional Polymers 2000, 46, 1-27.

8. Zhang Maogen, Conor Mullens, & Waldemar Gorski, Coimmobilization of dehydrogenases and their cofactors in electrochemical biosensors. Analytical Chemistry 2007, 79, 2446-2450.

9. Veerapur, R.S., Gudasi, K.B.& Aminabhavi, T.M., Pervaporation dehydration of isopropanol using blend membranes of chitosan and hydroxypropyl cellulose. Journal of Membrane Science 2007, 304, 102-111.

10. Gao Yunhua, Lee Kyue-Hyung, Mitsuko Oshima&Shoji Motpmizu, Department of Chemistry, F. o. S. O. U.; Department of Chemistry, F. o. S. O. U.: Adsorption Behavior of Metal Ions on Cross-linked Chitosan and the Determination of Oxoanions after Pretreatment with a Chitosan Column. Analytical Sciences 2000, 16.

11. Zeng Yan, Zhu Zhihong, Wang Ruixia & Lu Guanghan, Electrochemical determination of bromide at a multiwall carbon nanotubes-chitosan modified electrode. Electrochimica Acta 2005.

12. Lian Huiting, Sun Xiangying, Xu Jinrui & Wang Wenji, Development of a covalently bonded chitosan pH sensor. Analysis Laboratory.2007, 026, 100-103.

13. Li Jia, Xu Jinrui & Sun Xiangying, Determination of Nitrite by covalently bonded chitosan chemically modified electrode. Chinese Journal of Analytical Chemistry.2002, 030, 206-209.

14. Feng Jiuju, Zhao Ge, Xu Jingjuan & Chen Hongyuan, Direct electrochemistry and electrocatalysis of heme proteins immobilized on gold nanoparticles stabilized by chitosan. Analytical

Biochemistry 2005, 342, 280-286.

15. Bruno C. Janegitz, Luiz H. Marcolino-Junior, Sérgio P. Campana-Filhoc, Ronaldo C. Fariaa&Orlando Fatibello-Filhoa, Anodic stripping voltammetric determination of copper (II) using a functionalized carbon nanotubes paste electrode modified with crosslinked chitosan. Sensors & Actuators B Chemical 2009, 142, 260-266.

16. Liu Aihua, Itaru Honma& Zhou Haoshen, Amperometric biosensor based on tyrosinase-conjugated polysaccharide hybrid film: Selective determination of nanomolar neurotransmitters metabolite of 3,4-dihydroxyphenylacetic acid (DOPAC) in biological fluid. Biosensors & Bioelectronics 2006, 21, 809-816.

17. Wang Xueying, Deng Kejun & Tu Yifeng, Electrochemical study of electrodeposited Prussian blue/chitosan modified gold electrode. Analytical Chemistry.2008, 36, 632-636.

18. Celeste A. Constantine,Sarita V. Mello,Annie Dupont, Xihui Cao,David Santos, Jr.,Osvaldo N. Oliveira, Jr. Francisco T. Strixino, Ernesto C. Pereira, Tu-Chen Cheng, Joseph J. Defrank&Roger M. Leblanc, Layer-by-layer self-assembled chitosan/poly (thiophene-3-acetic acid) and organophosphorus hydrolase multilayers. J.am.chem.soc 2003, 125, 1805.

19. Tan Xuecai, Huang Tingting, Xu Jianjun, Luo Lijun, Zou Xiaoyong & Cai Peixiang, Preparation and characterization of chitosan/silica hybrid membrane with immobilized horseradish peroxidase. Chinese Journal of Analysis and Testing,2004, 23, 9-12.

20. A. H. CastroNet, F. Guinea, N.M.R. Perez, K.S. Novoselov & A.K. Game, Electronic Properties of Graphene (Minipgm). Reviews of Modern Physics 2009, 81, 109-162.

21. A.K.Game & K.S.Novoselov, The rise of graphene. Nature Materials 2007, 6.

22. Sasha Stankovich, Dmitriy A. Dikin, Geoffrey H. B. Dommett, Kevin M. Kohlhaas, Eric J. Zimney, Eric A.Stach, Richard D.Piner, SonBinh T.Nguyen& Rodney S. Ruoff, Graphene-Based Composite Materials. Nature 2006, 442, 282-286.

23. Guo Huilin, Wang Xianfei, Qian Qingyun, Wang Fengbin & Xia Xinghua, A green approach to the synthesis of graphene nanosheets. ACS Nano 2009, 3, 2653-2659.

24. Liu Sheng, Ou Junfei, Wang Jinqing, Liu Xiaohong & Yang Shengrong, A simple two-step electrochemical synthesis of graphene sheets film on the ITO electrode as supercapacitors. Journal of Applied Electrochemistry 2011, 41, 881-884.

25. Zhou Yige, Chen Jingjing, Wang Fengbin, Sheng Zhenhuan & Xia Xinghua, A facile approach to the synthesis of highly electroactive Pt nanoparticles on graphene as an anode catalyst for direct methanol fuel cells. Chemical Communications 2010, 46, 5951-5953.

26. Wang Yongqiu, Research on the application of screen-printed electrodes in electrochemical biosensors. Screen printing industry,2018, 000, 50-53.

27. Chen T, Zeng Baoqing, Liu J L, Dong J H, X Q Liu, Z Wu, X Z Yang & Li Z M, High throughput exfoliation of graphene oxide from expanded graphite with the assistance of strong oxidant in modified Hummers method. 2009.

28. Y William S. Hummers, JR.&Richard E. Offerman, Preparation of Graphitic Oxide. Journal of the American Chemical Society 1958, 80.