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Research Article

Research on Application of Electrochemical Immune Sensors in Food Safety Detection

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Abstract: Currently, research on toxic and hazardous substance in food immunological detection methods focused on conventional enzyme-linked immunosorbent assay, especially relatively few studies Array Detection of toxic foods for electrochemical immunoassay method of harmful substances. The work to build a variety of stable performance, high selectivity and sensitivity of electrochemical immunosensor and applied to the detection of Sudan, clenbuterol and chloramphenicol and other foods toxic and hazardous materials. And for the sample of the large number of food safety testing, testing and testing required to have many kinds of characteristics such as age, photo-electrochemical immune sensor array was prepared and applied to veterinary drug residues in food in a fast, high-throughput analysis.

Keywords: Detection, electrochemical immune sensors, food safety

INTRODUCTION

Immune sensor is based on antigen-antibody specific recognition function developed into a kind of biosensors. Electrochemical sensors are immune immunesensor research first, most species, but also a more mature branches. Electrochemical immunosensor traditional immunoassay techniques and electrochemical sensor technology as one solution, because of its fast speed, high sensitivity, good selectivity, small for online technology, etc., in the pharmaceutical industry, environmental protection and food safety testing has broad application prospects. The booming nanotechnology and in particular the emergence of applications with a variety of special properties of nanomaterials, also proposed for this area in the new test principle and testing technology, the development of new, sensitive immune sensors open up a new world (Wang, 2005).

With the change in consumer attitudes and increase China's economic growth and national income, health attitudes, food safety has become a focus of public attention, the relevant state departments of supervision of food safety issues are also increasing. But in recent years, "Sudan", "lean", "melamine" and other major food safety incident is still time to time. Serious harm to the health of consumers, to protect people's lives and safety, the development of rapid, sensitive and reliable food safety testing methods are currently the top priority of the work of many researchers. Traditional food safety testing techniques such as gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry and other methods to detect the presence of a long period, complicated operation, required expensive equipment and other shortcomings, it is difficult to meet the food in the rapid detection of toxic and hazardous materials needs. The electrochemical immunosensor for its high sensitivity, good selectivity analysis fast, simple equipment and other characteristics can be monitored in the field of food safety testing has broad application prospects in complex systems. This study was prepared based on a variety of nanomaterials electrochemical stability immune sensors and apply food safety testing. For a sample of the large number of food safety testing, testing and testing required to have many kinds of characteristics such as age, the present study was prepared photo-electrochemical immune sensor array and applied to veterinary drug residues in food fast, high-throughput analysis (Pumera *et al*., 2007).

The electrochemical immunosensor comprising a recognition element and converting means, characterized in the detection signal is an electric potential or current. When the receiver recognizes the substance to be detected by the immune response, the amount of chemical conversion with an electric signal and outputs a signal related to analyte concentration by converting means and then processed by a computer and displayed. The principle structure is shown in Fig. 1. Due to the antigen or antibody as the identification element of the sensor, the electrochemical immunosensor highly specific, rapid analysis complex

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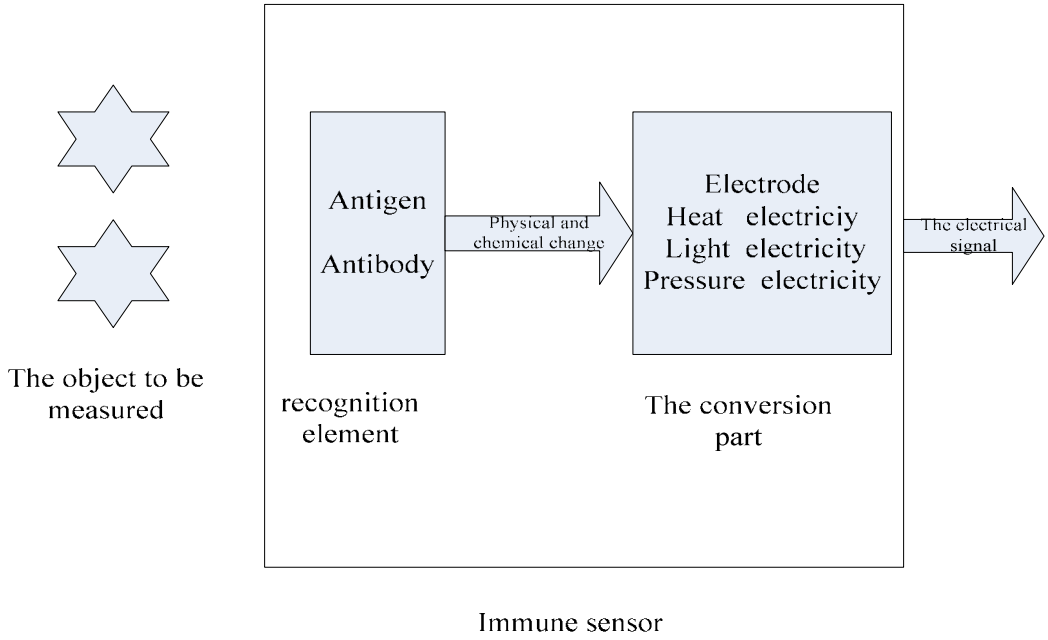

Fig. 1: Schematic diagram of electrochemical immunosensor

system to obtain the desired information analysis tools (Viswanathan *et al*., 2009).

METHODOLOGY

Electrochemical immunosensor theory:

Electrochemical immunosensor detection method: Electrochemical immunosensor detection methods include direct law, competition law and sandwich. Currently the most, the most simple method of operation is the direct method, the content of the system under test antigen (antibody) by the electrochemical reaction of the changing nature of liquid antigen (antibody) with direct binding antibody (antigen) is caused to reflect (Wang, 2006).

Competition law can be divided into direct and indirect competition law competition law. Direct competition law generally exists in two forms:

- Competition in antigen test electrode surface antigen binding enzyme (or other electroactive species) labeled antibody, electrical solution after adding the substrate changes.
- Antibody labeled antigen compete with the electrode surface and a known amount of the antigen binding, measured changes in the electrochemical properties of the electrolyte after addition of the substrate.

More difficult to prepare because of HRP, generally used immunoassay labeled secondary antibodies (enzyme-labeled or other electrically active substance mark) determination of the test substance in indirect competition law. Indirect competition law can be measured by a variety of common enzyme-labeled antibody analyte, eliminating the trouble of marking each antibody and increased sensitivity. After measuring electrical electrode surface antigen and the antigen competition test solution combines antibodies (antibody) was then added labeled secondary antibody binding antibody solution, adding the substrate: the indirect method of operation of competition law change in the signal. Competition law system can greatly improve the sensitivity analysis and better detection of small molecules results.

Sandwich assay refers to an antibody binding antigen in the sample with the electrode surface, then add labeled secondary antibody binding an antigen on an electrode, measuring an electric signal changes of the substrate solution was added to determine the amount of antigen. However, this method is only applicable to large molecules of antigen detection and quantitative analysis and not for the determination of small molecule hapten.

Classification of electrochemical immunosensor: The electrochemical immunosensor in accordance with the immunoassay process whether to use markers can be divided into: Type of non-labeled and labeled immunosensor; according to the kind of the measurement signal can be divided into: potentiometric, conductivity type, capacitance type and current type four. Where the current type of immune sensor is the most mature study, the most widely used one (Scognamiglio, 2013).

Preparation and its application based on the detection of Sudan I monoclonal antibody immune electrochemical sensors: Dan azo dyes (including Sudan I-IV Number) is a class of chemical synthetic dyes, mainly used for oil, enriched oil and some other industrial solvents, are also used for credit shoes, floor, etc. due to simultaneous its good coloring and long-term food bright appearance, Sudan has also been illegally used food coloring added to dried chili powder, chili medicine, medical and sour duck eggs and other foods. However, studies have shown that Sudan dyes are a class of humans and animals is a potential carcinogen, has been classified as a carcinogen IARC categories. Countries in the world have banned the pigment added to foods as its. Therefore, a rapid and reliable method for sensitive detection of Sudan's food is very necessary.

Experimental part:

Reagent: Carboxylated Sudan I (CSD I, Chuangwei biotechnology company, Guangzhou), Sudan I, II, III, IV No. 1-ethyl-(3-dimethylaminopropyl) carbodiimide salt salt (EDC), N-base amber light to Shoot imide (NHS), Bovine Serum Albumin (BSA), Ovalbumin (OVA), goat anti-mouse immunoglobulin-horseradish peroxidase (goat anti-mouse IgG-HRP), Freund's complete adjuvant and incomplete adjuvant, polyethylene glycol 4000 reagent was purchased from Sigma (USA). Hypoxanthine/Aminopterin/Thymus Jie secret instigate (HAT) and Fetal Calf Serum (FCS) was purchased from GIBCO biological reagents Company (NY, USA). Skimmed milk powder purchased from BIO Basic Company (NY, USA). Balb/c mice were purchased from Shanghai experimental Animal Center Chinese Academy of Sciences (Shanghai). RPMI 1640 was purchased from Invitrogen (USA), o-thiol acid (0-MBA), Dicyclohexyl Carbodiimide (DCC), (HPLC grade N, N-Dimethyl amine ugly (DMF), methanol), Tween-20, 30% of 02 and 3, 3', 5, 5'-Tetramethylbenzidine (TMB) were purchased from Sinopharm reagent Company (Shanghai). All other reagents were of analytical grade and no further treatment, the experimental double-distilled water (Luo *et al*., 2006).

Instruments and equipment chemistry experiment CHI660d electrochemical analyzer (CH instruments, USA); using a three-electrode system: Immune sensor as the working electrode, a gold electrode diameter is 3 mm (Tokai Corporation); Gan beam Saturation Electrode (SCE) as reference electrode; pin glass electrode as the counter electrode. Raman spectroscopy (RamTracer-200-WF-I, Suzhou); UV-visible spectrophotometer (Varian, Cary50Conc). Liquid Chromatography (LC10AT, Shimadzu), UV detector. ELISA assay using 96-well polystyrene microtiter plates (COSTAR, USA), EXL800 plate reader (BIOTEK, United States).

Preparation of samples: Three kinds of dried chilli powder samples (samples A, B and C, respectively) were purchased from local markets. Respectively, in accordance l0, 20 and 50 g/g concentrations to the sample A, B and C, respectively Sudan I added standard samples spiked sample preparation. All samples were dried and mixed well. Weigh l.0 g dried chili powder sample, small, soluble l0 mL DMF sonicated 30 min, 4500 rpm centrifugal analysis l0 min. The supernatant was stored for use.

RESULTS AND DISCUSSION

By way of a peptide bond, carboxylated Sudan I is bonded on top of a carrier protein to produce an immunogen. Figure 1 and 2 CSD I, BSA and CSD I-BSA complex UV spectrum. Shown, BSA UV spectral chart showing a characteristic absorption peak at 280 nm at, CSD I UV spectrum exhibits a characteristic absorption peak at SlOnin place, while the UV CSD I - BSA complex spectra in at 280 and 310 mn presents two characteristic absorption peaks, the results show that the CSD I have been successful and BSA conjugate prepared immunogen.

Preparation immunosensor: Preparation of immune sensor diagram is shown in Fig. 3. First, MBA by strong gold-sulfur bond bonding self-assembled monolayer film formed on the gold electrode surface. To ensure that the electrode surface of the fixed high concentration of antibodies to obtain a high sensitivity and good reproducibility of the measurement, the reaction followed by NHS-EDC-activated self-assembled layer of the electrode surface generate NHS and then the antibody of the primary amine groups substituted with NHS cool groups, antibodies cool amide bond formation is immobilized on the electrode

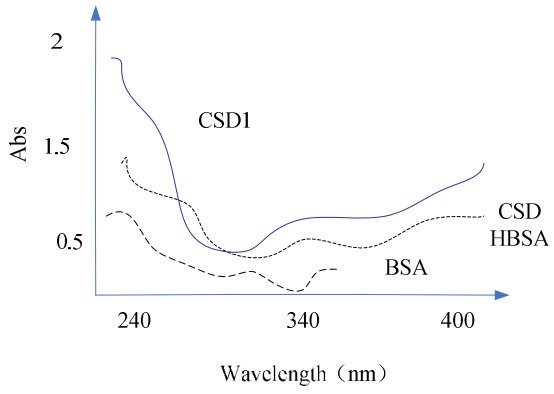

Fig. 2: UV-vis spectra of CSD I, BSA and CSD I-BSA

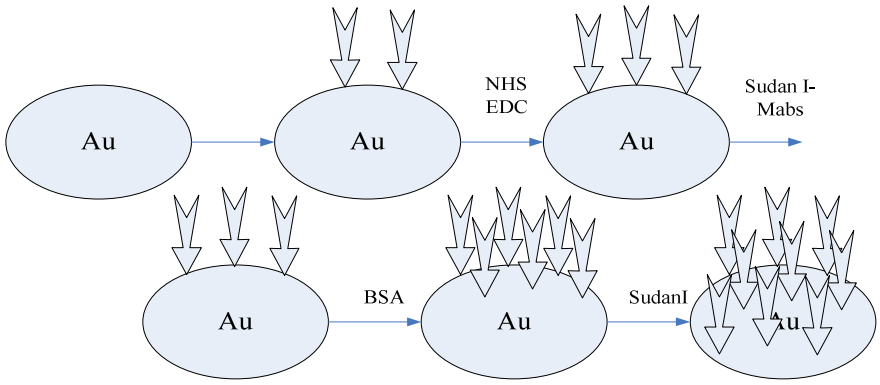

Fig. 3: Schematic illustration of the stepwise immunosensor fabrication process

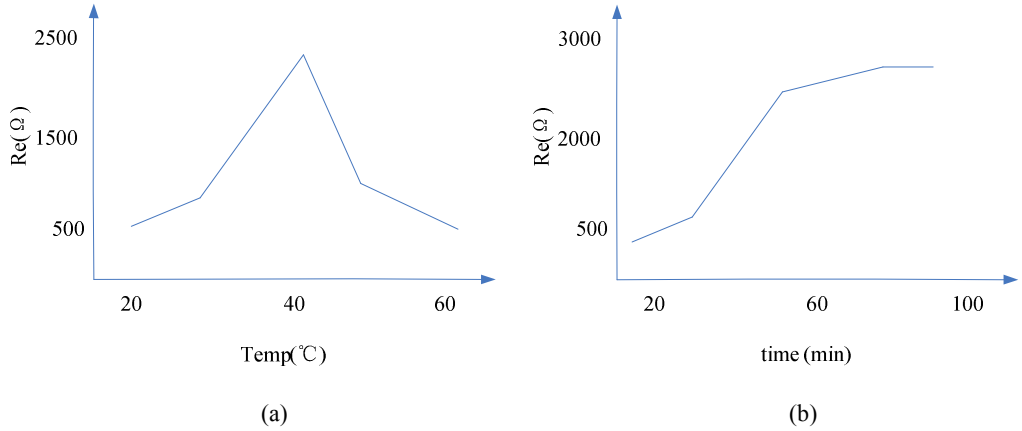

Fig. 4: Effect of (a) Incubation temperature, (b) Immuno-reaction time

surface. Finally, the immune response to the antigen by the antibody and antigen Sudan I will bind to the surface of the immunosensor.

Sudan I in specific immune sensor surface combination will change the nature of the electrode surface and cause changes in electron transfer impedance. This paper studied the incubation temperature and time on the electrode surface temperature electron transfer impedance.

Experiments examined the effects of the range of 20-6°C incubation temperature on the immune value of Ret. Impedance response as shown in Fig. 4 maximum at 37°C, while at temperatures above 37°C impedance response values decline. This may be because incubation at temperatures exceeding 37°C may cause the antigen-antibody complex decomposition and degeneration irreversibly react. While the temperature is below 37°C will reduce the immune reaction rate, reaction time. Therefore, we select 37°C as the optimal incubation temperature.

The incubation time will significantly affect the immune response to an antigen and antibody. As shown, the impedance response with increasing the reaction time increases 4, when the reaction time reaches 60 min, impedance response stabilized. Thus, the experiment to select the optimal incubation time for 60 min (Vlakh and Tennikova, 2013).

Preparation of CU@AU nano-particles labeled immune electrochemical sensors and applied to the detection of clenbuterol-based: Clenbuterol (CB), commonly known as "lean", is a typical 6-receptor agonist, was originally used for the treatment of asthma and premature children. Because of its anabolic steroids and similar promote muscle growth and reduce body fat content of the physiological role of being illegally used as growth promoters in livestock word compound added to improve livestock meat production. But the drug is easy in livestock body (especially the liver) deposition, human consumption of meat containing residues of the drug can cause acute poisoning symptoms after. In recent years, poisoning "lean" triggered often found in countries reported. Therefore, China, the European Union and many countries have banned the CB as a growth promoter for livestock feed. To protect public safety, exploitation of meat products CB quick, effective and sensitive detection method is very urgent.

CB is currently the most widely used detection methods include Gas Chromatography-Mass

Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC)-Electrochemical detection and mass spectrometry and Capillary Electrophoresis (CE). Although these methods can more accurately detect the residual amount of the CB, but tedious sample preparation method, long analysis period. And the equipment is expensive, difficult to meet the general needs of researchers. In recent years, the analyst has developed such electrochemical detection method (Freire *et al*., 2003), Enzyme-Linked Immunosorbent Assay (ELISA), surface plasmon resonance sensors, electrochemical immunoassay method is more rapid and simple detection method CB. Because both the immune wherein the electrochemical sensor electrochemical analysis equipment is simple, low cost of detection, high sensitivity and high traditional immunoassay specific advantages and concern. Since most of the antigen and antibody are electrochemically inert, electrochemical immunoassay typically requires the use of notation, the indirect determination of the analysis concentration measured by the amount of the marker. Therefore, selection marker substance directly affects the sensitivity of immunoassay. In order to improve the sensitivity of immunoassays, researchers developed a radioisotope, enzymes, fluorescein and metal nano-particles such as antibody markers, greatly expanded the scope of application of immunoassay (Zhang *et al*., 2005a).

In recent years, the metal nano-particles because of their unique optical and electrical properties in the immune analysis has been widely used. Compared with a single metal nano-particle, composite nano-particles are more suitable antibodies in immunoassays as a marker. Such as gold nano-particles prepared by simple, stable performance and good biocompatibility of antibodies, but its relatively poor electrical activity. Based on this, the synthesis of the core-shell Cu@Au nano-particles and CB labeled monoclonal antibody used. Compared with the single gold nano-particles, core-shell Cu@Au nano-particles in the outer layer of gold nano-particles with good biological compatibility and stability and to protect the inner layer of Cu, making it less susceptible to oxidation, while the inner layer nano-crystalline Cu has excellent electrochemical activity, which was used as the antibody marker significantly improves the electrochemical detection sensitivity (Zhang *et al*., 2005b).

In this study, we will CB molecule is covalently bound to the CMWCNTs, then CB-CMWCNTs composite dropped onto the glassy carbon electrode surface to form a stable CB-CMWCNTs film and finally the Cu@Au labeled antibody specifically binding CB to construct composite membrane constructed competitive CB electrochemical immunosensor.@Copper gold nano-particles to form Cu_2^+ dissolution, using GC/Nafion/Au markers to determine the concentration of copper in the modified electrode by Anodic Stripping Voltammetry (ASV), thereby indirectly measuring the content of the competition clenbuterol. Competition CB concentration detection system, the smaller the amount of the sensor surface binding of labeled antibody, Cu dissolution without reducing the value of the current relative time current competitive CB (AI) greater. The results show that, AI and competitive CB concentration of values in the 0.1? 1000 μg/mL linear range, detection limit of 0.05 μg/mL (3 sec), this method is used to detect liver samples spiked CB's sample recovery in 94.5? between 104.3% and ELISA was highly correlated with the recovery. All results show that the method is rapid, sensitive, reliable and can be extended to the immunological analysis of other samples.

Experimental part:

Instruments and reagents clenbuterol hydrochloride, Bovine Serum Albumin (BSA), Horseradish Peroxidase (HRP): Goat anti-mouse IgG were purchased from Sigma. (0.5%) from 5% Nafion solution prepared by diluting ethanol. Clenbuterol monoclonal mouse anti-antibody was purchased from Hangzhou Lungi biotechnology company. Carboxylated multi-walled carbon nanotubes were purchased from Chinese Academy of Sciences, Chengdu Institute of Organic Chemistry. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDO). N-through thio cool Shot glass imide (NHS) was purchased from Shanghai Sinopharm Chemical Reagent Co., Sulfate pentahydrate Copper ($CUSO_4.5H_2O$), four water alloy Acid ($HA_{11}CI_4.4H_2O$), sodium Borohydride ($NaBH_4$), pick acid Levin (Hg (N_{03}; h), oxygen Bromate (HBr), perchloric acid ($HCIO_4$), etc., other chemical reagents were purchased from Shanghai chemical Reagent Co. reagents used were analytical reagent pure without further purification, experimental water is deionized water Phosphate Buffer Solution (PBS) by 0.2 inoli;.. iNa_2HP_{04} and NaH_2P_{04} solution configuration, with 1.0 mol/L NaOH solution to adjust pH = 7.4.

Cary 500 UV-visible spectrometer (Varian Comp, United States): High resolution transmission electron microscope image and an electron diffraction data collected from JEOLJEM-2010TEMgEOI ^, Japan). ELISA analysis was performed using a 96-well polystyrene microplate (COSTAR, USA). Microplate reader EXL800 purchased in BIOTEK (Winooski, VT, USA). All electrochemical experiments were carried out in CHI660a electrochemical analyzer (CHI, USA).

Anodic stripping voltammetry preclude the use of a three-electrode system: a working electrode for GC/Nafion/Hg modified electrode, SCE Levin electrode as reference electrode, drill glass electrode as

the counter electrode. Preparation GC/Nafion/Hg modified electrode: glassy carbon electrode (<I) = 3 min) with particle size 0.5 μm oxide yue polished to a mirror trick on suede, Steam Museum washout washed sequentially in acetone, acid sales (1: 1), sodium hydroxide solution (50%, w/w) and distilled water ultrasonic cleaning pregnant 5 min, take 10 μL 0.5% of Nafion solution droplets coated glassy carbon electrode surface clean, dry and then placed in a natural 0.1 mol/L NaAc-HAc solution detector cell containing l00 mg/L Levin ion deposition potential at -1.0 V 600 sec, Joan dry spare.

Sample preparation: Liver samples were purchased from a local supermarket. Respectively 10 μg/g, 20 μg/g, concentration 50 μg/g of the liver samples A, B, C were added CB liver spiked sample prepared standard sample. Each weighed l0.0 g, according to documents prepared by the liver sample extract, placed 4°C refrigerator for use.

Principle of electrochemical immunoassay: Firstly, CMWCNTs by EDC and NHS-activated amine formation ugly cool and then form a peptide bond with clenbuterol in CB-CMWCNTs amine to form complexes (Fig. 5a), CB-CMWCNTs finally formed on the glassy carbon electrode stable modified membrane. When the electrode is immersed in a competitive CB antibody and a mixed solution, CB-CMWCNTs competition on CB solution and the electrode solution free of monoclonal antibody, as shown in Fig. 3 to 5b, the greater the competition CB concentration in the sample, binding to markers electrode fewer, Cu_2^+ dissolution response current will be lower. After washing away unbound labeled antibody, bound antibody markers of Cu@Au nano-particles was dissolved in the acid solution is formed of gold and copper ions, copper ions released by ASV deposited on GC/Nafion/Hg electrode, Cu dissolution current corresponding linear relationship between the concentration of clenbuterol competing values into.

Optimized immunoassay conditions: Incubation temperature and time are important factors immunoassay. This study examines the 20-6 (rc within the scope of the effects of different incubation temperature on the dissolution of the current response of copper results 3-3A shown in the current response of the maximum at 37°C, the temperature is above 37°C as shown later response current also decreases with increasing temperature may be due to this high temperature will occur such as antibodies and antigen-antibody complexes decomposition and degeneration irreversible reaction, thus affecting the immune response In addition, a temperature lower than 37 T will slow down the immune reaction rate, reaction time, so the experiment was 37°C to select the optimum incubation temperature.

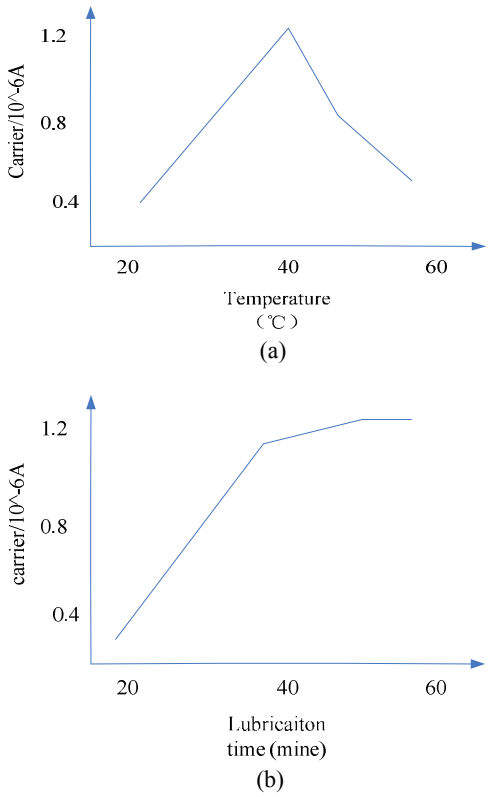

Fig. 5: Effect of (a) Incubation temperature, (b) Immuno-reaction time

Influence of incubation time on the dissolution of the current shown in Fig. 5b. With increasing reaction time, the peak current value gradually increases, after a stable value over 60 min tends, therefore the experiment to select for 60 min incubation time.

CONCLUSION

Electrochemical immunoassay immune sensor and electrochemical sensor technology combined with small size, high selectivity and sensitivity, short response time, less required sample characteristics. Electrochemical immunosensor in the pharmaceutical industry, environmental monitoring and food safety testing and many other fields have broad application prospects. The nano-technology, especially after various nano-materials with special properties applied to the field of electrochemical sensors immunity, not only improve the detection performance of the sensor and the sensor so that all aspects of nature as well as their biological macromolecules or small molecule detection sensitivity significantly increase, the detection time can be shortened and can achieve real-time detection of high-throughput analysis.

This study focuses on nanotechnology and electrochemical immunoassay technology, has developed a novel electrochemical immunosensor based on nano-materials will be applied to foods

representative of Sudan poisonous substance, clenbuterol and detection of chloramphenicol and to explore the mechanism of its detection.

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