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Application of Electrochemical Immuno-sensor Based on Ketamine Hydrochloride for the Detection of Sports Illicit Drugs

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Abstract: *With the development of modern technology, the developed electrochemical immuno-sensor becomes a new kind of micro measurement technology. The electrochemical immuno-sensor based on ketamine hydrochloride was prepared in this study. Sufficient antibodies are combined with the electrode through adsorption of 3-mercaptopropionic acid and gold electrode. The results showed that the electrode had a specific response to antigens. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and atomic force microscope (AFM) were used for the electrode characterization. Results indicated that, the prepared electrode had good stability and reproducibility. The prepared electrochemical immuno-sensor based on ketamine hydrochloride in this study was applied to the detection of morphine content in sports illicit drugs, and we found that, such kind of sensor was appropriate for detection of illicit drugs. Moreover, it had high sensitivity and was hopeful to be made into a miniature instrument. Thus, the electrochemical immuno-sensor based on ketamine hydrochloride plays an important role in the detection of illicit drugs on the competition site of sports events.*

Keywords: ketamine hydrochloride; electrochemical immuno-sensor; sports illicit drugs; morphine; sensitivity

1. INTRODUCTION

The biosensor has strong sensitivity, especially to low-concentration chemicals or biochemicals, and it is accurate and effective in detection, thus it has important application in the field of medicine and bioanalysis. Moreover, the rapidity and convenience of the biosensor play a key role in its efficiency detection. As a kind of biosensor, immuno-sensors have good sensitivity and selectivity to antibodies and antigens, which are mostly applied to the field of medical detection [1-2].

Electrochemical immuno-sensors have plenty of functions, but they are mostly applied to the detection of bioactive substances due to their good sensitivity. Low-toxic or nontoxic reagents are usually adopted during the detection using electrochemical immuno-sensors. Therefore, they must be less harmful to human and environment compared with other methods. Moreover, the manufacturing process of the electrochemical immuno-sensors is simple and convenient and low-cost and they are appropriate for field detection. Accordingly, they are extensively applied to detection of illicit drugs in sports events [3-4].

Ketamine hydrochloride (KTHC) is usually used as the anesthetic to stimulate nervous centralis, which is a kind of colorless and odorless chloride. Morphine, as a kind of alkaloid extracted from opium, is a kind of drug that can inhibit nervous centralis, which is harmful to human health and is one of the most abused drugs in the world [5-6]. Pretreatment methods used in detection of morphine in sports illicit drugs include solid-phase microextraction, solid phase extraction and liquid-liquid extraction, which are simple and convenient [7]. Methods used to detect morphine are varied, such as chromatography, immunoassay, spectroscopy and electrochemical sensor method. Because electrochemical immunosensors have advantages like rapidity and efficiency, high sensitivity and accurate analysis results, etc., their application in drug examination has become one of the research emphases in this field [8-9]. The electrochemical immuno-sensor based on KTHC made in this study not only has high sensitivity and small size, but also is barely influenced by the sample matrix, which can save the time of sample pretreatment; moreover, the electrochemical immunosensor has good reproducibility and stability, thus can be applied in practical detection effectively.

2. DETECTION OF KTHC USING THE IMMUNO-SENSOR

2.1. Experimental facilities and reagents

Experimental facilities used in this study include RST5200 elec-

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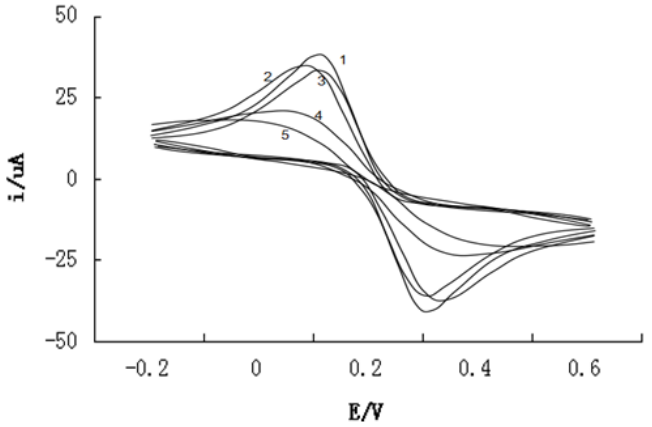

Figure 1. Cyclic voltammogram (curves of (1) bare gold electrode, (2) 3-mercaptopropionic acid modified, (3) EDC-NHS activation, (4) Antibody firstly modified by 20ìL of KTHC and (5) Sufficient modified antibodies)

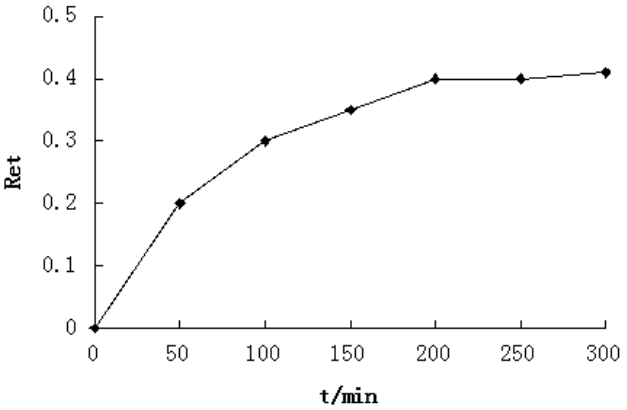

Figure 2. Influence of the time of the EDC-NHS activation on the change transfer resistance of the reaction

trochemical workstation from Rui Si Te Instrument Corporation of Suzhou City, atomic force microscope from American Bruker Corporation, drying oven, ZSimpwin software and three-electrode system from Jin Hong Instrument Corporation of Shangshai. Reagents used in this study include 3-mercaptopropionic acid, N-Hydroxysuccinimide(NHS), 1-ethyl-3(3dimethylaminopropyl)-carbodiimide (EDC), KTHC, mouse monoclonal antibodies, KTHC injection and bovine serum albumin (BSA), etc.

2.2. Antibody immobilization

(1) Gold electrodes purchased from TianJin AIDhengsheng Science-Technology Development Limited Company were polished and washed by potassium dichromate; after that, the gold electrode was washed ultrasonically by ethyl alcohol and deionized water respectively for 10-15 min, and the process was repeated for three times.

(2) The washed gold electrode was put into sulfuric acid (1 mol/L) for activation, and then activated electrode was put into potassium ferricyanide supporting electrolyte solution for electrochemical impedance spectroscopy (EIS) scanning. After that the electrode was dried and reserved.

(3) After the nude gold electrode was soaked in 3-mercaptopropionic acid for 12 h, it was washed by absolute ethyl alcohol for three times and then dried under nitrogen atmosphere. Then the electrode was immediately put into the mixture of EDC and NHS for 4 h for activation. The electrode was then taken out and washed by ultrapure water for three times and then dried again under nitrogen atmosphere.

(4) An amount of 20 ìL of KTHC was dropped onto the gold electrode and then the electrode was incubated at 36 °C for 60 min; the process was repeated once until an adequate number of antibodies were modified.

2.3. Detecting steps of KTHC

(1) Serum samples were added into KTHC in different concentrations to obtain antigen serum samples in different concentrations.

(2) Prepared serum with different concentrations of antigen was added into the supporting electrolyte solution of a three-electrode system for EIS examination; response results were recorded.

(3) Serum samples were diluted and the interference from matrix was eliminated; then serum samples were applied to the detection of KTHC.

2.4. Analysis of experimental results

2.4.1. Effect of different modified electrodes on electron transfer

Figure 1 shows that, the current peak values and their potential position could change the number of layers of modification and they might affect the electron transfer process. The peak current of curve 1 was small and there was a long section of relatively straight line in curve 1. In curve 2, the negative valence ionic groups of the 3-mercaptopropionic acid were on the surface of modified electrode, thus the electron transfer was impeded. After the EDC-NHS activation, the resistance value of curve 3 increased, indicating that EDC-NHS successfully replaced the carboxyl of 3-mercaptopropionic acid; the peak current of curve 4 decreased when the antibody were fixed on the electrode, which was due to the impedance of the electron transfer caused by the adsorption of ketamine antibody on the surface of the electrode. Finally, the resistance value increased even more due to the combination of ketamine antigen and antibody specificity, as curve 5 shows.

2.4.2 Optimization results analysis of experimental conditions

Mainly the time of EDC-NHS activation, the number of binding antibodies and the temperature of antibody modification in this study received experimental optimization.

As shown in figure 2, the resistance value of the electron transfer increases with the accumulation of time. When the time reaches 250 min, changes of the resistance value of electron transfer becomes less significant, indicating that the EDC-NHS has successfully replaced the carboxyl of 3-mercaptopropionic acid. Thus, 250

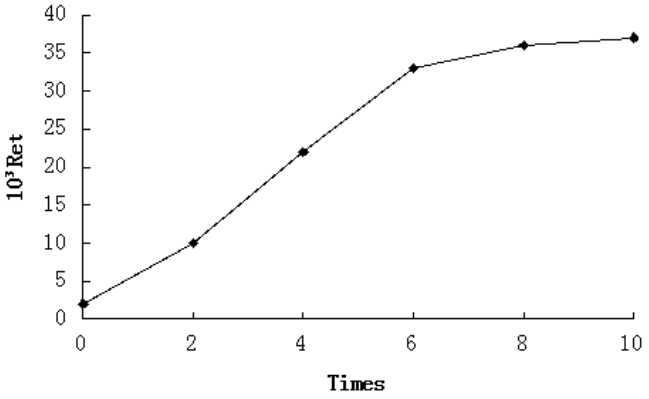

Figure 3. Effect of the times of binding antibodies on the resistance of electron transfer due to the detection of antigens

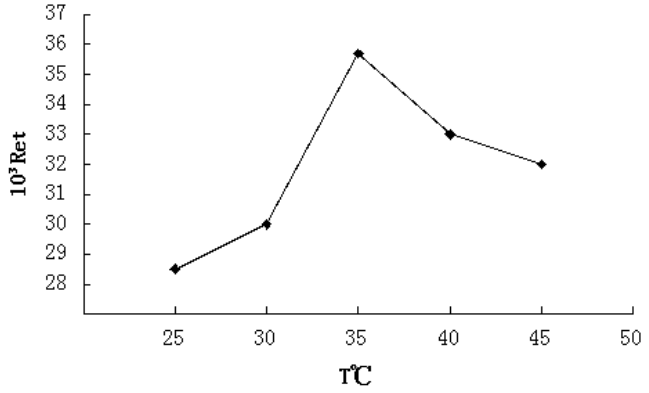

Figure 4. Effect of the temperature of antibody modification on the resistance of electron transfer due to the detection of the antigen

min was the ideal optimization time.

As shown in figure 3, the influence of the times of binding antibodies on the detection of antigens is very important. When the time reaches 10 min, the resistance value reaches maximum and sufficient antibody molecules have been fixed on the surface of electrode.

Figure 4 indicates that, when the temperature was at 35 °C, the maximum resistance value could be obtained by modifying antibodies onto the electrode.

2.4.3. Stability and reproducibility of the KTHC immuno-sensor

Stability: prepared immuno-sensor was blown dry by nitrogen and preserved at 4 °C. It was taken out everyday for stability test for seven days. The results showed that the resistance value did not change significantly with the accumulation of time, indicating that the electrode had good stability [10].

Reproducibility: prepared immuno-sensor was blown dry by nitrogen and preserved at 4 °C; its resistance value was detected everyday and no significant changes were observed, indicating that it had good stability. After four times of elution, only a few residuals were found. Thus the influence of accumulation time on the functional performance of the immuno-sensor was little and the KTHC immuno-sensor had good reproducibility [11].

3. DETECTION OF SPORTS ILLICIT DRUG MORPHINE USING IMMUNO-SENSORS

3.1. Sports illicit drug – morphine

3.1.1. Definition of morphine

Morphine (MOP) is an important part of opiate drugs and its content in opium accounts for about 10%. Morphine hydrochloride, a common kind of anesthetic used in clinic, has powerful analgesic effect and is often used for pain relieving for athletes in sports events; besides, it is a kind of sports illicit drugs, which should be detected by precise instruments [12].

3.1.2. Pharmacological effects of MOP

(1) MOP has strong anaesthetic and analgesic effect on the central nervous system, which can eliminate emotions like anxiety, tension and fear, etc., caused by pain, thus it can enhance patients' endurance to pains [13].

(2) MOP has soothing and antitussive effect, which can restrain activities of the respiratory center and coughing center in brain and thus slow down and relieve the intense breath of patients. MOP is mostly used medically.

(3) A large dose of MOP can lead to postural hypotension and bradycardia, making the body feels uncomfortable.

(4) MOP also has excitatory effect, especially to the smooth muscle and sphincter of the gastrointestinal tract; taking MOP can lead to tension increase of the smooth muscle and sphincter of the gastrointestinal tract, which can weaken the peristalsis, thus MOP also has anti-diarrhea and anti-constipation effect [14].

3.1.3. Detection methods of MOP

(1) Chromatography: chromatography has high accuracy and sensitivity in analyzing MOP, which is appropriate for detection of the trace amount of MOP in samples.

(2) Spectroscopy: spectroscopy can be used for simultaneous field identification of MOP contents in multiple drugs, which has powerful functions and is highly efficient, rapid, harmless and environmentally friendly Meanwhile, it can use near-infrared technology to detect the content of MOP in whiting non-destructively, and its testing range is wide.

(3) Immunoassay: currently, it has been a common method to use immunoassay to detect small molecules. Current immunoassays that can be used for detection of MOP include radioactive immunoassay and enzyme-linked immunoassay, etc.

(4) Electrochemical sensor method: the method of using electrochemical sensors is simple, convenient, time-saving and highly sensitive. It can be used alone or as the detector of chromatography. Currently, it is a most commonly used method [15].

3.2. Detection of MOP in serum using electrochemical immuno-sensors

(1) MOP antigen was added into the blank serum to prepare the serum sample.

(2) Serum samples with different concentrations of MOP anti-

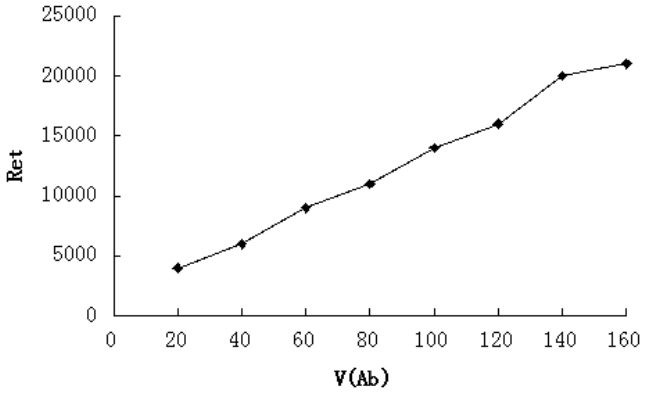

Figure 5. Effect of the concentration of the binding antibodies (20 μL per time) on the resistance of the electron change transfer

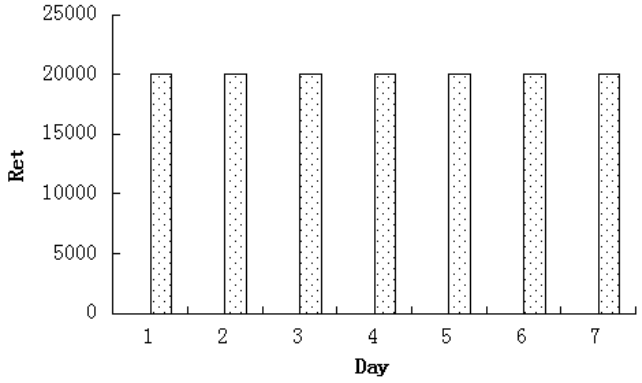

Figure 7. Effect of the testing time on the resistance value due to MOP reaction using immuno-sensors

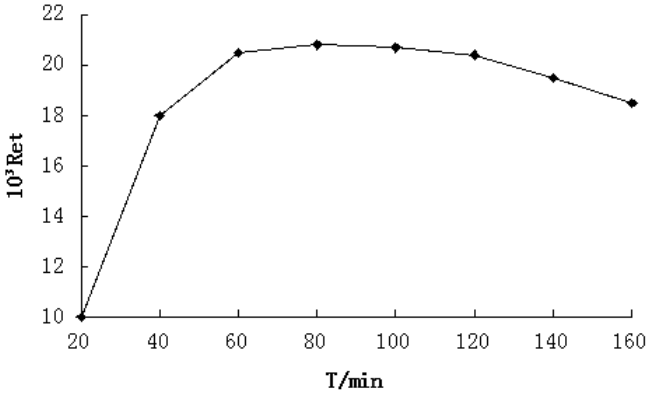

Figure 6. Effect of the time of antibodies binding on change transfer resistance of the MOP antigen

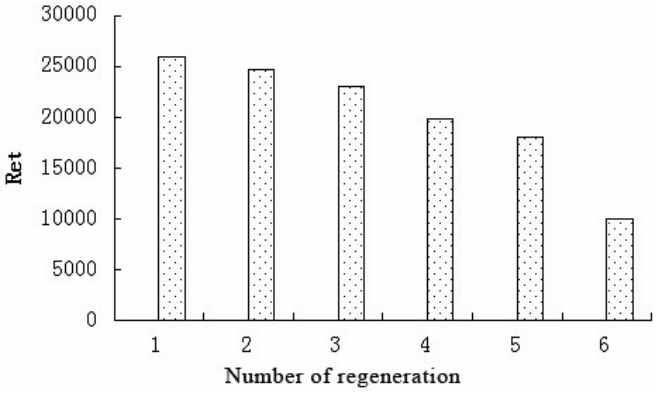

Figure 8. Variation of the resistance of the electron transfer resistance of the separation of antigen from antibody complex with the number of regeneration

gen were added into the supporting electrolyte solution of three-electrode system for EIS measurement.

(3) Impedance responses of electrode to serum samples with different concentrations of antigen were recorded.

3.3. Optimization of experimental conditions

The number of binding antibodies and the time of binding antibodies have been mainly optimized in this study.

Figure 5 indicates that, the resistance value increases from the initial 4000+Ω to 20000+Ω until no significant changes occur, suggesting that sufficient antibody molecules have been fixed on the electrode surface of sensor at that time.

As shown in figure 6, in the humid environment at 30 °C, high resistance values can be obtained when the time of binding the antibodies was between 60 and 100 min.

3.4. Detection of stability and reproducibility of MOP using immuno-sensors

3.4.1. Analysis of results of stability test

The same as the reproducibility experiment of the KTHC immuno-sensor showed before, the resistance values of the electrode were tested for continuous seven days and the results showed that the resistance values had no significant changes (figure 7), indicating that the electrode had good stability.

3.4.2. Analysis of results of reproducibility test

The electrode was soaked in NaOH-H_3PO_4 solution for 5-7 min, thus to separate antigen from antibody complex (figure 8). Such kind of immuno-sensors used to detect MOP can reproduce at least 4-5 times, which indicates its good reproducibility.

3.5. Analysis of testing results of MOP in urine samples

Urine samples can be obtained more easily in real life and their matrix is much simpler compared with blood samples; results of urine test have higher accuracy. Urine samples in this study were diluted to 1000 times to eliminate the effect of matrix, thus the interference was small. Therefore, good results can be obtained simply by 1000 times of dilution (figure 9).

4. DISCUSSION

Nowadays, various kinds of illicit drugs are circulated in illicit market, such as heroin and morphine, etc., which have resulted in

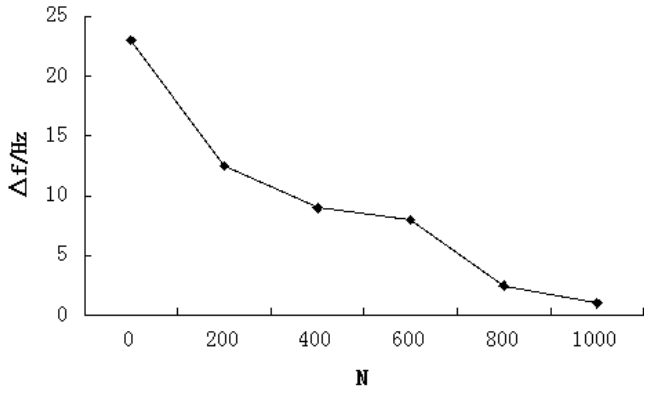

Figure 9. Effect of dilution ratios on the detection

severe influence and harmful effects on socio-economic development, stability of people's life as well as the health of human body worldwide. In order to increase the detection range of these drugs as well as to reduce the limit of detection, this study adopted the KTHC electrochemical immuno-sensor to detect the sports illicit drug – morphine, thus to achieve the possibility of fast field detection [16].

Firstly, self assembled technology was adopted to modify the surface of electrode and antibodies were connected through EDC-NHS activation; finally, morphine was fixed on the surface of electrode to prepare the morphine immuno-sensor, and such sensor was applied for the detection of sports illicit drugs. The sensor had good reproducibility and stability, and its operation was easy and fast, which provided reliable reference for fast field detection [17-18]. In addition, morphine was introduced briefly and its detection methods were deeply analyzed; EIS was adopted for optimization of experimental conditions, and obtained electrochemical immuno-sensor based on KTHC was used to detect the content of morphine in serum and urine samples. Experimental results showed that, the sensitivity of such kind of sensor was very high [19-20]. In order to avoid the interference from matrix in urine samples, urine samples were diluted to 1000 times, which could save a lot of time caused by pretreatment of samples using chromatography-mass spectrometry method [21-23].

In conclusion, the application of electrochemical immuno-sensor based on KTHC in detection of sports illicit drugs is effective, and such kind of electrochemical immuno-sensor has high sensitivity as well as good performance and rapid response. Moreover, the pretreatment of the samples is very simple and convenient, which provides a good platform for realization of fast and accurate field detection; meanwhile, it can be preserved easily and it can save costs and time in practical detection.

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