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Short Communication

Electrochemical Behavior of Salbutamol, Clenbuterol, Ractopamine and Albuterol at CNTs/GCE

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Salbutamol, clenbuterol, ractopamine and albuterol have the effect of promoting protein synthesis and increasing muscle mass. Despite that these substances are banned or restricted in animal husbandry in many countries, some farmers still use them illegally to improve profits. Athletes can test positive for stimulants after ingesting food with these drug residues. In this study, the electrochemical behavior of salbutamol, clenbuterol, ractopamine and albuterol were investigated with carbon nanotubes modified glassy carbon electrodes. The linear detection range and detection limit of four stimulants were studied under optimal conditions.

Keywords: Athletes; Stimulants; Electroanalytical chemistry; Agonists; Anabolic steroids; Glucocorticoids; Gibberellins

1. INTRODUCTION

Athletes of endurance events in sports tend to run or exercise for days without rest, so athletes in swimming, cycling, long-distance running and race walking often use stimulants such as strychnine, heroin and amphetamines to combat fatigue. However, with the increasing occurrence of adverse reactions, governments and sports organizations have realized that doping not only violates the sportsmanship of fair competition, but also brings a lot of harm to the athletes' health [1–5]. Since 2004, the world anti-doping organization has issued a series of regulations to prohibit athletes from taking any stimulant drugs, and the list of banned stimulants is updated every year. More than 265 banned or restricted drugs are on the 2016 list. Nowadays, few athletes take the risk of doping with the strengthening of anti-doping propaganda and education as well as the intensive and strict doping control [6–9]. However, in recent years, athletes' doping positive cases for the reason of taking food have still been common in sports events. In order to shorten the feeding cycle, increase the survival rate and reduce the cost, many farmers abuse antibiotics, protein assimilators and hormones and other drugs in the

livestock feeding process, resulting in high concentrations of banned drugs in many animal-derived foods [10–15]. Among all the food-borne stimulants, clenbuterol is the most difficult to guard against. In practice, the proportion and content of clenbuterol in meat on the market are much higher than the safety standard of meat for athletes, and the positive proportion of pig, beef, mutton and offal in meat is much higher than that in other kinds of meat [16–20]. Therefore, the detection of food-borne stimulant is one of the important content of athletes' daily management services.

Receptor agonists are a class of drugs that can selectively bind to corticoadrenergic receptors to produce receptor agonists. Agonists are the first and most common drugs used to treat asthma [21–27] and they are also widely used in chronic obstructive pulmonary disease and a variety of respiratory diseases. It has been found that receptor agonists can promote protein synthesis, reduce fat synthesis and deposition, and increase ketone lean meat rate in animals. However, high doses of agonists left in animals can pose health risks to the animals themselves and their consumers. As a result, both the European Union and China have banned the use of these substances in livestock production. Receptor agonists are a series of derivatives with phenylethanolamine being the parent nucleus, aromatic ring and terminal amino group. Detection of receptor agonists in animal-derived foods is challenging due to the large number of homologues with low concentrations. Initially, radioimmunoassay and enzyme-linked immunoassay were adopted in many countries as control methods of receptor agonists. However, due to the influence of antibody extraction and crosslinking activity, radioimmunoassay and enzyme-linked immunoassay cannot be used to confirm receptor agonists [28–33]. In recent years, an increasingly extensive attention has been paid to the high selectivity and rapidity of immunoassay with the application of surface plasma resonance technique, high efficiency thin layer chromatography and affinity chromatography. However, official inspection institutions tend to choose mass spectrometry methods with high sensitivity, high specificity and good repeatability for both screening and confirmation [34–38].

Despite that liquid chromatography has advantages over gas chromatography in the separation of agonists without requiring derivatization, and is more compatible with solvents used in chromatographic systems, effective liquid chromatography-mass spectrometry systems for routine testing were not developed until the 1980s, when gas chromatography-mass spectrometry was the only alternative. Clare et al. applied GC-MS in terbutaline test in 1979 [39]. In 2010, Wang et al. [40] successfully detected salbutamol, clenbuterol, ractopamine and albuterol in meat, liver and kidney with GC-MS, and the detection limit was as low as 0.04 ~ 0.09 ug/kg. As an alternative to GC-MS, LC-MS has been repeatedly adopted to detect receptor agonists in samples such as urine, plasma, liver, animal chicken, etc. The first biological analysis of receptor agonists with LC-MS was carried out in 1989. Blanchnower and Kennedy applied this method to the detection of clenbuterol residues in cow urine [41]. Kootstra et al. [42] detected cimatro and other 8 receptor agonists in beef with LC-MS in 2004, and it has been verified that this method is applicable to substrates including rabbit meat, duck meat, turkey and various kinds of fish. With the development of ion trap-time of flight series mass spectrometer, the application scope of LC-MS has been further expanded, and the detection quantity and sensitivity have been gradually improved. Sai et al. [43] have recently adopted HPLC-LIT-MS for 25 agonists and 23 receptor blockers detection in pork, chicken, liver and other animal-derived foods.

However, the GC-MS and LC-MS analysis method requires a complicated experimental process with an expensive instrument. Compared with the two methods, electrochemical method has the advantages of good stability, high sensitivity, cheap equipment, low price, high degree of automation and easy miniaturization, being a good choice for the detection of agonists in field food [44–50].

Common commercial electrodes have limited sensitivity and selectivity for direct detection of phenol, thus the surface modification of commercial electrodes has become a common method to improve the performance of electrodes. Carbon nanotubes (CNTs) discovered by Iijima in 1991 are the most characteristic one-dimensional nanomaterials, with a length of microns and a diameter of nanometers, as well as extremely high aspect ratio and super mechanical properties [51–58]. The application of CNTs has involved many aspects such as nano-electronic devices, catalyst carriers, electrode materials, hydrogen storage materials and composite materials. Scientists call CNTs the "super fiber" of the future for their unique properties [59–63]. CNTs play a number of roles in composite materials with these excellent properties, for instance, the super mechanical properties can greatly improve the strength and toughness of composites, and the unique conductive and photoelectrical properties can enhance the conductivity of polymer materials and prepare new photopolymer composites [64–69]. In this work, acidified CNTs have been adopted for the surface modification of glassy carbon electrode (GCE). The prepared CNTs/GCE have been adopted for electroanalytical determination of salbutamol, clenbuterol, ractopamine and albuterol.

2. EXPERIMENTAL

Salbutamol, clenbuterol, ractopamine and albuterol were purchased from Shanghai Aladdin biochemical technology Co. Ltd. CNTs were obtained from Xilong Chemical Co. Ltd. A certain amount of CNTs were placed into a three-necked bottle at different concentration ratios, and about 30 mL concentrated sulfuric acid (H_2SO_4) and 10 mL concentrated nitric acid (HNO_3) were added. Afterwards, the three-necked bottle was placed into an ultrasonic wave for ultrasonic treatment for 30 min. The purpose of this step was to preliminarily disperse the CNTs and make the hard agglomerations between the molecules open and become soft agglomerations. The above ultrasonic CNTs were stirred at 50^{o}C, and then stopped heating. They were rapidly cooled to room temperature under ice water to prevent the re-oxidation reaction. Centrifugation was adopted for water washing until it was neutral, after which it was taken out and put on the dish to dry for 24 h.

The above prepared CNTs were then dispersed into water to reach a concentration of 0.5 mg/mL. Afterwards, a certain amount of dispersion was dropped on the GCE surface and dried naturally. The modified electrode was denoted as CNTs/GCE. All electrochemical determination was conducted at a CHI760E working station. A Pt wire and a Ag/AgCl (3 M KCl) were applied as counter electrode and reference electrode, respectively. Electrochemical impedance spectroscopy (EIS) was recorded in 5 mM PBS containing 5 mM Fe(II)/Fe(III) as redox moieties (pH = 7.4). The EIS tests were performed in the frequency range between 10^{-2}–10^{5} Hz. The CV measurements were proceed with the scan rates of 50 mV/s.

3. RESULTS AND DISCUSSION

The morphology of the CNTs has been observed with SEM. As shown in Figure 1A, it can be seen that the acidified CNTs show clear aggregation. Electrochemical impedance spectroscopy (Nyquist plots) is an effective method to describe the resistance of electron transfer at electrode interfaces. It can be divided into the high-frequency region and the low-frequency region. The radii of the arc part of the high-frequency region represent the resistance of electron transfer, and the linear part of the low-frequency region represent the resistance of material diffusion. As shown in Figure 1B, the electrochemical impedance spectrum of the bare GCE has a much larger arc in the high-frequency region, indicating that the electrode surface has a high electron transfer resistance. The electrochemical impedance spectrum of CNTs/GCE has a small arc in the high-frequency region, indicating that the acidized CNTs can significantly improve the conductivity.

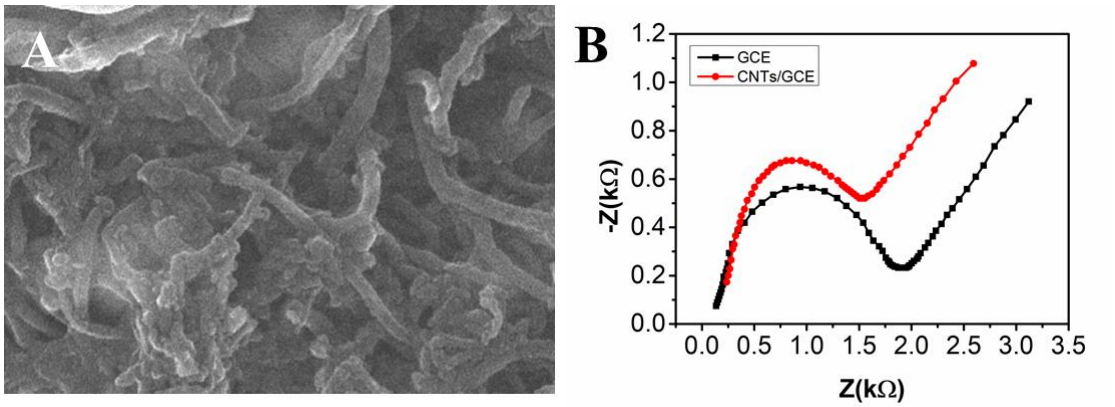

Figure 1. (A) SEM image of CNTs (B) Electrochemical impedance diagram of GCE and CNTs/GCE.

In order to investigate the electrochemical behavior of different modified electrodes with the same concentration of salbutamol, clenbuterol, ractopamine and albuterol, the CV diagrams of GCE and CNTs/GCE in a solution containing 10 μM of salbutamol, clenbuterol, ractopamine and albuterol were shown in Figure 2. It can be seen that the bare GCE shows no responses towards salbutamol and clenbuterol. Small peaks can be observed for ractopamine and albuterol at 1.51 V and 0.83 V, respectively, suggesting both ractopamine and clenbuterol can be oxidized at bare GCE. In contrast, four stimulants all exhibit distinct electrochemical responses at CNTs/GCE surface. Specifically, the electrochemical oxidation of salbutamol, clenbuterol, ractopamine and albuterol are found at 1.17 V, 0.82 V, 1.22 V and 0.87 V, respectively. This enhancement can be ascribed to the high conductivity of CNTs. In addition, the acidified CNTs can provide many surface defects for triggering the electroanalytical responses when an electrochemical reaction takes place. The result of salbutamol detection is consistent with the CV results reported by Rajaji et al. [70], who proposed a novel binary nanosheets of Bi_2Te_3/GCN nanocomposite via facile approach for salbutamol detection. The result of

ractopamine detection is interestingly consistent with the EIS results reported by Orooji et al. [71], who reported an electrochemical sensor for ractopamine detection.

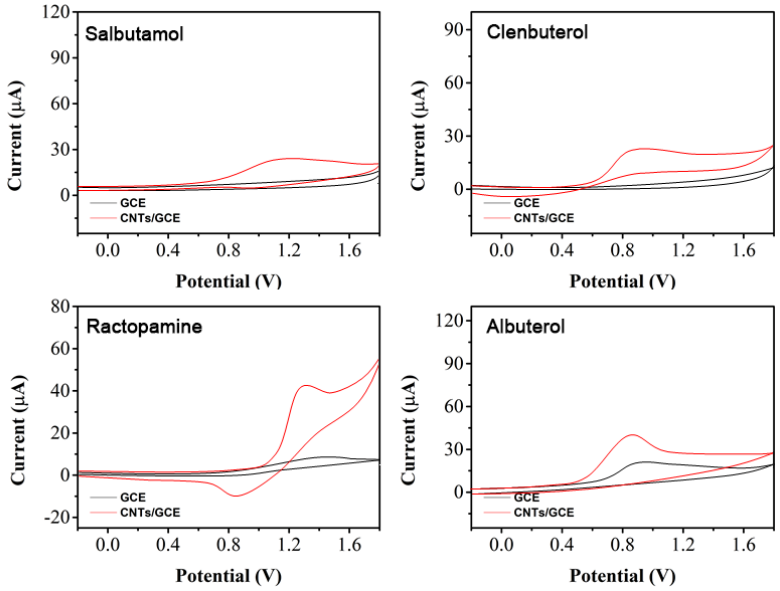

Figure 2. Cyclic voltammetry curves of GCE and CNTs/GCE towards 10 μM of (A) salbutamol, (B) clenbuterol, (C) ractopamine and (D) albuterol.

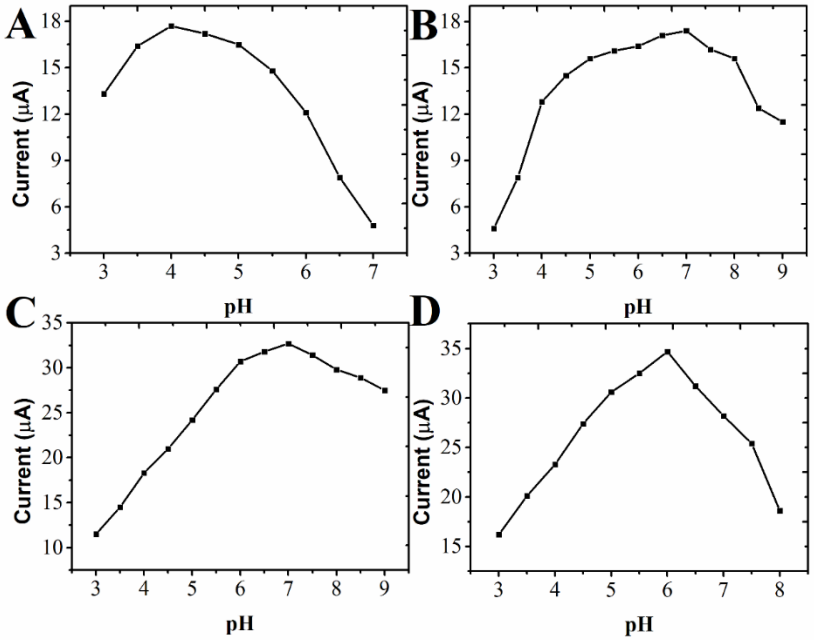

Figure 3. Effect of pH condition on (A) salbutamol, (B) clenbuterol, (C) ractopamine and (D) albuterol sensing.

The experiment conditions have been optimized. Figure 3A shows the plot of the effect of pH condition on salbutamol oxidation. It can be noted that the pH of 4 shows the best sensing performance towards salbutamol oxidation, which indicates that an irreversible reaction, or an electrochemical

reaction is followed by a chemical step [72]. Figure 3B shows the plot of the effect of pH condition on clenbuterol oxidation. It can be seen that the pH of 7 presents the best sensing performance towards clenbuterol oxidation. Figure 3C shows the plot of the effect of pH condition on ractopamine oxidation.

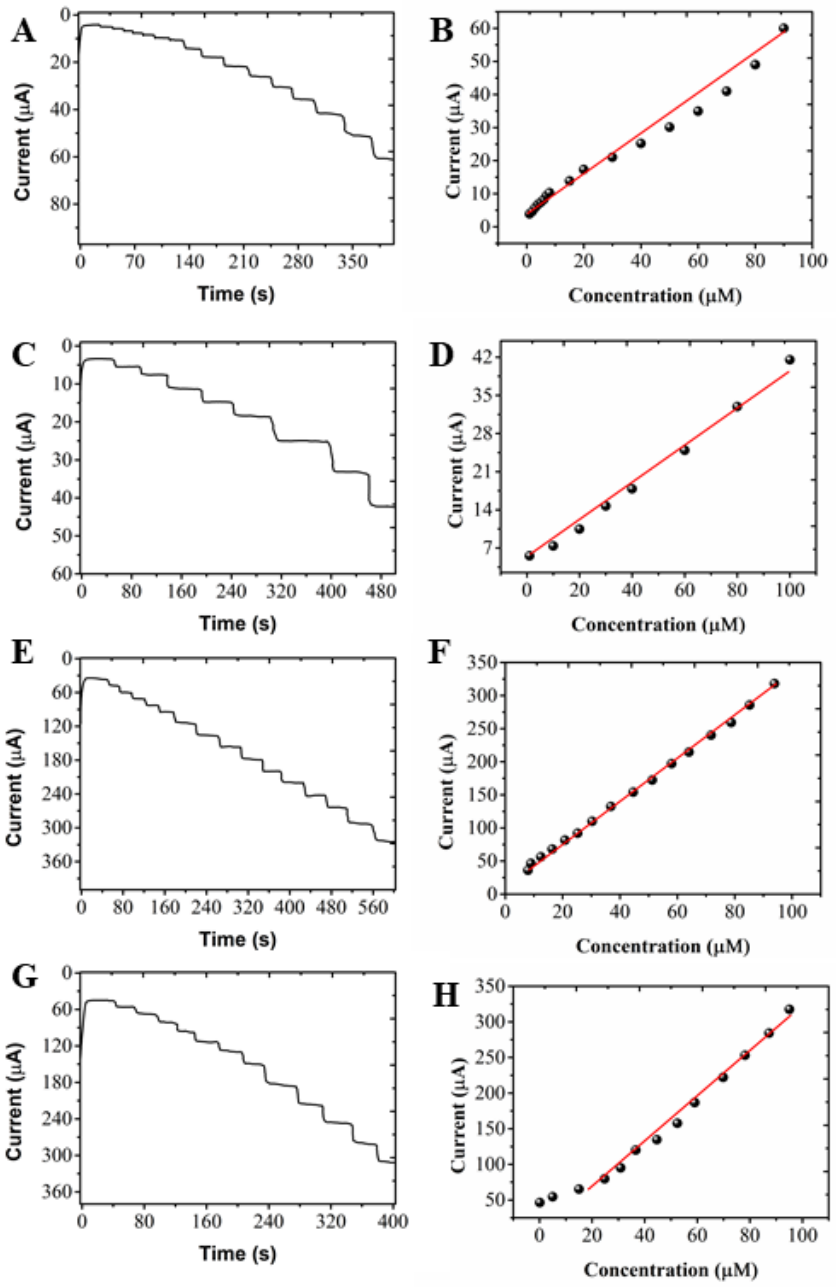

Figure 4. (A) I-T curves of CNTs/GCE with different amount of salbutamol added. (B) Plot of concentration of salbutamol vs. current response. (C) I-T curves of CNTs/GCE with different amount of clenbuterol added. (D) Plot of concentration of clenbuterol vs. current response. (E) I-T curves of CNTs/GCE with different amount of ractopamine added. (F) Plot of concentration of ractopamine vs. current response. (G) I-T curves of CNTs/GCE with different amount of albuterol added. (H) Plot of concentration of albuterol vs. current response.

It can be seen that the pH of 7 shows the best sensing performance towards ractopamine oxidation. Figure 3D reveals the plot of the effect of pH condition on albuterol oxidation. It can be noted that the pH of 6 shows the best sensing performance towards albuterol oxidation.

The analytical performance of the CNTs/GCE towards different concentration of salbutamol is shown in Figure 4A, from which it can be found that the current response increases immediately with the introduction of salbutamol solution and reaches a stable state within 3s. The relationship of current responses and salbutamol concentrations can be found in Figure 4B, which presents a linear relationship between current responses and salbutamol concentrations from 1 μM to 90 μM. A limit of detection can be calculated to be 0.35 μM based on the S/N=3. Figure 4C shows the I-T curves with the addition of different concentrations of clenbuterol. A linear relationship can be found between current responses and clenbuterol concentrations from 1 μM to 100 μM (Figure 4D). A limit of detection can be calculated to be 0.27 μM based on the S/N=3. Figure 4E shows the I-T curves with the addition of different concentrations of ractopamine. A linear relationship can be found between current responses and ractopamine concentrations from 1 μM to 0.1 mM (Figure 4F). A limit of detection can be calculated to be 0.34 μM based on the S/N=3. Figure 4G shows the I-T curves with the addition of different concentrations of ractopamine. A linear relationship can be found between current responses and ractopamine concentrations from 10 μM to 0.1 mM (Figure 4H). A limit of detection can be calculated to be 0.77μM based on the S/N=3. Table 1 shows the comparison of the sensing performance of this work with previous reports.

Table 1. The comparison analysis of analytical methods for the determination of salbutamol, clenbuterol, ractopamine and albuterol.

Sensor	Linear range	LOD	Reference
Salbutamol			
Graphene/PEDOT: PSS	1 nM to 1.2 μM	0.1 nM	[73]
Ag/Pd	0.01 to 100 ng/mL	1.44 pg/mL	[74]
Ag/N co-doped RGO	0.03 μM to 20 μM	7 nM	[75]
CNTs/GCE	1 μM to 90 μM	0.35 μM	This work
Clenbuterol			
MoS_2-Au-PEI-hemin	31 nM to 6.39 μM	6.13 nM	[76]
KVB-Nf (IP)/FCE	0.95 μM to 143 μM	0.75 μM	[77]
ZnSQD@PANI	0.0319 nM to 31.9 nM	0.0175 nM	[78]
CNTs/GCE	1 μM to 100 μM	0.27 μM	This work
Ractopamine			
RGO	25 μg/L to 1 mg/L	56 nM	[79]
Mesoporous carbon	0.085 μM to 8.0 μM	60 nM	[80]
AuNPs/PDDA-GN	1 pM to 10 nM	0.5 pM	[81]
CNTs/GCE	1 μM to 100 μM	0.34 μM	This work
Albuterol			
PPA/MWCNT/GCE	50 nM to 70 μM	12 nM	[82]
AgNP-IL-FG-NF/GCE	79 nM to 2.9 μM	17 nM	[83]
CNTs/GCE	1 μM to 100 μM	0.77 μM	This work

4. CONCLUSION

In this study, an electrochemical sensor based on acidified CNTs modified electrode was prepared, and electrochemical detection tests of four stimulants were conducted under the three-electrode system. Acidified CNTs have excellent electrocatalytic and electrical properties, which can catalyze the oxidation of stimulants. Under the optimal conditions, the proposed sensor could linearly detect salbutamol, clenbuterol, ractopamine and albuterol.

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