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Highly Sensitive Electrochemical Sensor based on Carbon Dots Reduced Gold Nanoparticles for Ractopamine Detection in Pork Meat

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A microwave synthesis process was used to successfully prepare carbon dots (CDs). Then the prepared carbon dots were employed as reductants to prepare gold nanoparticles. The kinetic characteristics of CDs@Au/GCE, the electrochemical behaviour of ractopamine (Rac) on CDs@Au/GCE and the electrochemical determination of Rac were investigated. Electrochemical characterization results show that the prepared gold nanoparticles can be used as a stable modification on the surface of a glassy carbon electrode (GCE). Differential pulse voltammetry (DPV) results show that within a range of 0.01 to 32.5 mg/L, the linear correlation coefficient of the response current to the Rac concentration is 0.9999, furthermore, the detection limit is 1.2 μg/L, which is lower than previous report (1.5 μg/L). The electrochemical sensors for Rac were developed using CDs to reduce gold nanoparticles. The above sensor have high sensitivity and good reproducibility and stability, thus, they show potential applications for Rac analysis.

Keywords: carbon dots, gold nanoparticles, ractopamine, electrochemical sensors, microwave synthesis

1. INTRODUCTION

In recent years, the abuse of Rac as feed additive has become a serious social problem [1, 2]. Consumers face serious health complications, such as heart and central nervous diseases if they eat meat form Rac-treated animals [3, 4, 5]. Therefore, it is urgent to create and apply a simple, fast, effective and low-cost analysis method for the determination of Rac, especially those can be handled in situ[1,3,6,7,8]. As far as we know, various methods, including HPLC, LC-MS and GC-MC have been employed in the determination of Rac [1, 6, 9, 10]. Unfortunately, many techniques are harmful, time-consuming, and labour-intensive; additionally, they often require highly educated personnel or sophisticate dinstrumentation, which make them unsuitable for field analysis and rapid screening [1]. Compared with the above methods, electrochemical methods area simple pretreatment procedures that have fast analytical responses and low cost instruments, thus, they show great potential for Rac analysis applications [3, 7].

Nanomaterials have unique properties, such as a large specific surface area, good catalytic activity, and unique chemical and physical properties. Because of these characteristics, they are used in electrochemical sensors and consumer products, and they are also used in the healthcare industry [11, 13]. In 1973, Bond et al. found that gold nanoparticles can simplify the hydrogenation process of olefins [13].Later, especially in recent years, gold nanoparticles have attracted attention due to their unique physical and chemical properties, including a high greater electrical conductivity, large specific surface area, variable particle sizes and morphologies, and strange optical properties. A variety of gold nanoparticles have been established as electrode modifiers and electrochemical sensors for different toxic compounds [14, 15]. For example, Kong et al. created a technique to determine Rac with a gold oly-o-aminothiophenol-modified gold electrode as a molecularly decorated quartz crystal microbalance (QCM) sensor [14]. Duan et al. fabricated a nano-chain structure of gold nanoparticles for Rac sensing [13]. Recently, our group constructed an ordered mesoporous carbon electrode modified witha flower-like gold nanostructure and applied it for the electrochemical determination of Rac, whose detection limit was much lower than that in existing reports [16].

Since its discovery in 2004, CDs have been used in various processes, such as SERS, bio-imaging, sensing, laser LED and photo-catalysis [17, 18, 19]. In the present work, CD- reduced gold nanoparticles were prepared. Using the as-prepared nanoparticles, an electrochemical sensor for Rac determination was constructed and evaluated. The results show that the sensor possesses a the low detection limit, wide linear range, and good reproducibility and stability, which indicates its potential application for food security evaluation.

2. EXPERIMENTAL PART

2.1 Reagents

From Fluka, Rac hydrochloride was purchased. The phosphate buffer was prepared using NaH_2PO_4 and Na_2HPO_4. Chloroauric acid was purchased from Shanghai Chemical. Co.Ltd. Carbamide and citric acid were obtained from Tianjin Chemical. Co.Ltd. All the purchased chemicals were at least analytical reagent grade. Pork samples were purchased from a Wal-Mart shopping plaza (Ginza Plaza), Changchun, China. All aqueous solutions were prepared with double-distilled water and kept at 4°C.

2.2 Apparatus

All electrochemical experiments were carried out using a CHI660C electrochemical workstation (Chenhua, China) in a conventional three-electrode cell. The modified GCE (diameter 3 mm), a piece of platinum wire and an Ag/AgCl electrode were used as the working, counter and reference electrodes, respectively. An APES microwave chemistry work station was obtained from Shanghai Yirao Co. Ltd.

2.3 Preparation of CDs

CDs were synthesized through the following procedure: First, 3.0g carbamide and 3.0g citric acid were dispersed in 10 mL H_2O, and then this solution was heated to 150°C in a 750 W microwave oven for 30min to obtain a brown product. Finally, the solution was centrifuged (3000 rpm) for 20min and then separated by a bag filter (8000-14000) for three days to remove large or agglomerated elemengts. After being freeze dried, solid CDs were obtained.

2.4 Synthesis of gold nanoparticles

CDs-reduced gold nanoparticles (Au@CDs) were prepared according to Shi's method [20]: 100 mL of $HAuCl_4$ (0.55 mg/mL) aqueous solution and 2 mL CDs (0.02 mg/mL) aqueous suspension were mixed together and kept at 100°Cfor 80 min to obtain a purple suspension.

For comparison, gold nanoparticles were also prepared with a traditional method [21]. After being heated to boiling, 25 mL of $HAuCl_4$ (0.01% by weight) were mixed with 375 mL of a sodium citrate aqueous solution (1% by weight).After are action time of 30 min, a wine-red suspension was obtained.

3. RESULTS AND DISSCUSSION

3.1 Characterization of CDs and Au@CDs

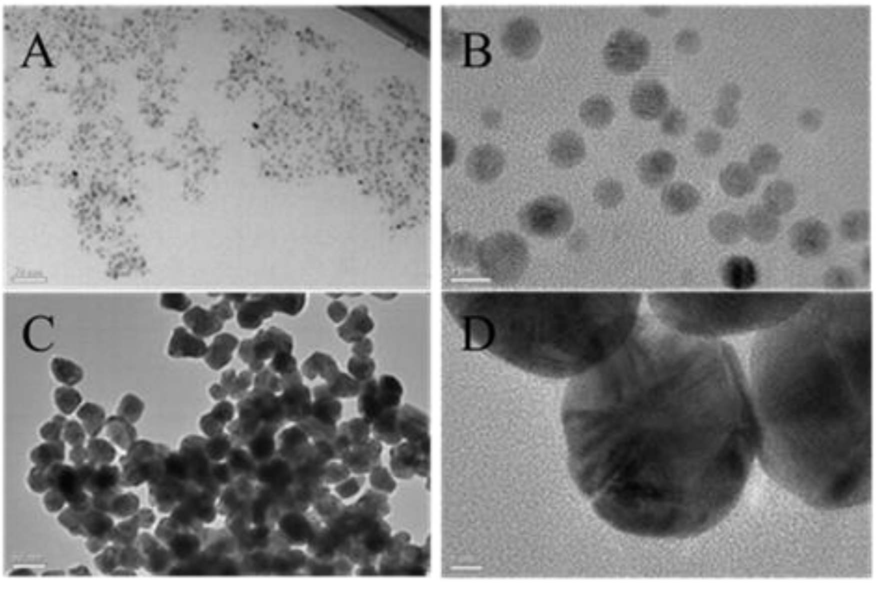

Figure 1. TEM images of CDs (A, B)**.** TEM images of Au@CDs (C, D).

Figure 1 A and B show the TEM images of the CDs. We can see that the size of the CDs are not entirely uniform. Most of the CDs are 5nm in diameter and are distributed between 1.1 nm and 1.9 nm. Figure 1 C and D show the TEM images of Au@CDs. It was found that the present material displays a spherical shape with a uniform particle size and a diameter distribution of 27±3 nm. The Elemental analysis (performed on a Flash EA1112 Elemental Analyzer) result showed that the C content of the Au@CDs is 22.56, which indicated the presence of CDs in the Au@CDs.

3.2 Kinetic characteristics of CDs@Au/GCE

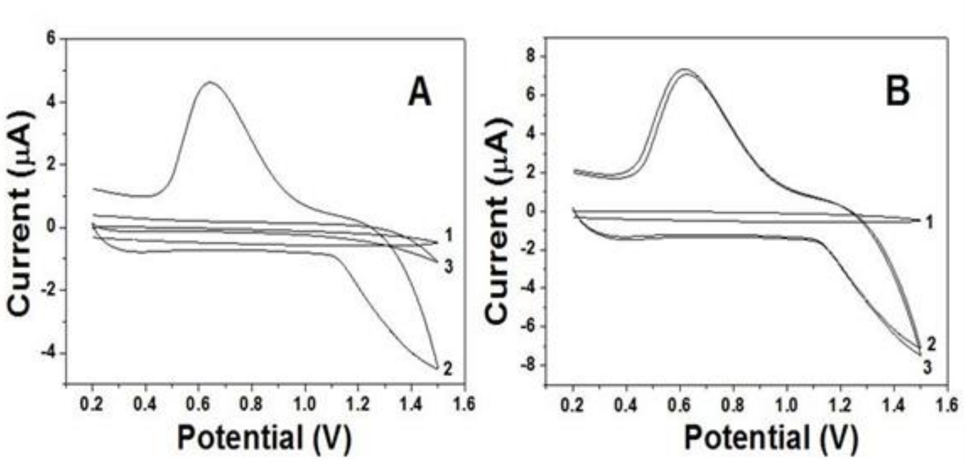

Figure 2. Cyclic voltammetry (CV) curves of GCE (A1,B1), gold nanoparticles/GCE (A) and CDs@Au/GCE(B) in a BR buffer solution (pH 2.0) after the first cycle (A2, B2) and after the thirtieth cycle (A3, B3). The scan rate was 50 mV/s.

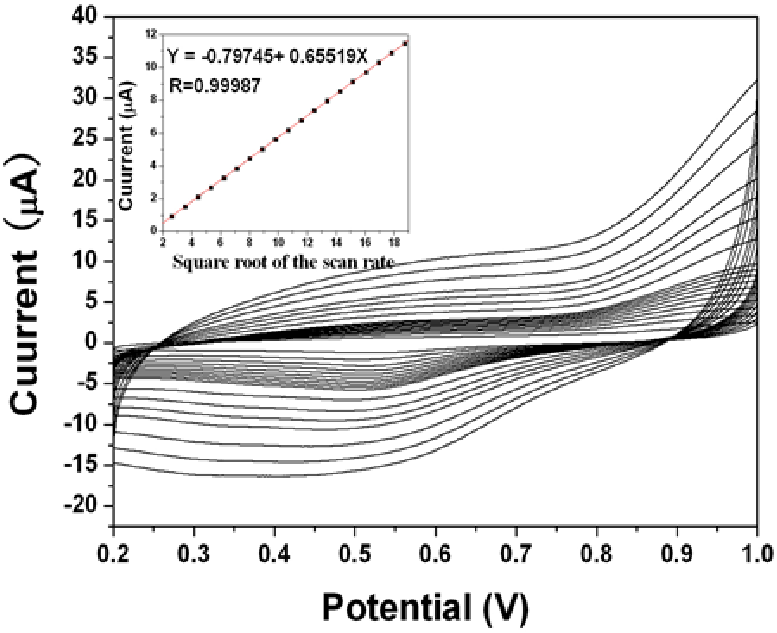

Figure 3. CVcurves of CDs@Au/GCE in 0.2mg/L Rac in PBS (pH 7.0) with scan rates of 10,20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 and 300 mV/s (from inner toouter). Inset: plot of the reduction peak current vs the square root of the scan rates.

Figure2 reveals the CV curves of GCEs modified with various species in a pH 2.0 BR buffer solution [15]. When gold nanoparticles are employed as the GCE modifier (Figure2A), a reduction peak at 0.662 V can be clearly seenin the first cycle, which is consistent with the characteristic electrochemical behaviour of gold and proves the successful modification of gold nanoparticles onto the GCE surface [21]. However, with an increasing number of cycles, the current response decreases and almost disappears after the 30th cycle, proving that the direct modification of pure gold nanoparticles is not stable [17]. When using CDs@Au as an electrode modifier (Figure2B), the situation changed. In the first cycle, similar to the gold nanoparticle-modified electrode, the characteristic electrochemical behaviour of gold is also observable. However, with an increasing number of cycles, the current response remains unchanged, revealing the stable modification of CDs@Au on the GCE surface [16].

Figure 3 shows the cyclic voltammetry curves of the CDs@Au/GCE-modified electrode in a PBS (pH7.0) solution with 0.2 mg/L Rac. It is found that with an increasing scanning speed, the redox behaviour of the CDs@Au-modified electrode to Rac increases gradually, that is, the peak current increases gradually [12]. Further analysis and study of the sweep rate and cyclic voltammogram show that when the sweep rate changes in a range of 10-300mV/s, the peak current is linearly related to the square root of the sweep rate (inset in Fig. 3) [17], and the correlation coefficient is 0.9998, indicating that the reduction process of Rac on the trim CDs@Au electrode is controlled by diffusion in the above sweep rate range [16].

3.3 Electrochemical behavior of Rac on CDs@Au/GCE

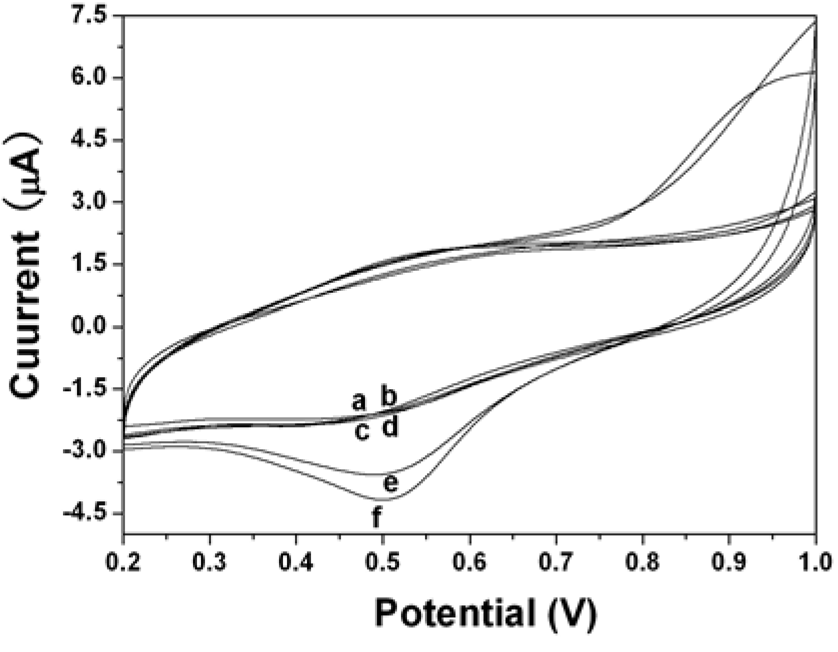

Figure 4. CV curves of GCE (a,b), CDs/GCE (c,d) and CDs@Au/GCE (e,f) in the absence (a,c,e) and presence (b,d,f) of 0.2 mg/L Rac in a PBS (pH 7.0) solution.The scan rate was 50mV/s.

The electrochemical behaviour of Rac was studied using CV in 0.2 mol/L PBS (pH 7.0). Figure 4 shows the CV curves obtained for the bare GCE (a, b), CDs/GCE (c,d)， and CDs@Au/GCE (e, f) in the absence (a, c, e) and presence (b, d, f) of 0.2 mg/L Rac. It is found that regardless of whether Rac is present, both GCE and CDs/GCE demonstrate no observable response, which indicates that the modification of CDs onto the GCE surface exhibits no electro-catalytic ability towards the electrochemical redox of Rac [17]. When CDs@Au is modified on the GCE, an obvious electrochemical response between 0.2 and 1.0 V and a reduction peak potential at approximately 0.5 V is observed. Upon the addition of 0.2 mg/L Rac, an enhanced reduction current occurs, which reveals the electro-catalytic reduction of Rac on CDs@Au/GCE [22].

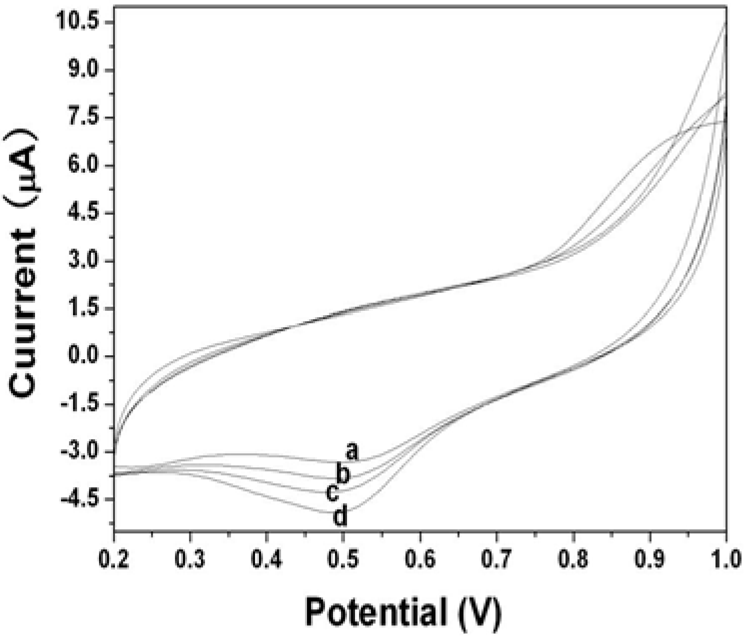

Figure 5. CV curves of CDs@Au/GCE in a PBS (pH 7.0) solution with 0, 0.12, 0.22 and 0.36 mg/L Rac (from a to d, respectively).The scan rate was 50 mV/s.

The CV responses of CDs@Au/GCE in 0.2 mol/L PBS (pH 7.0) in the presence of Rac with various concentrations are shown in Figure 5. It can be clearly seen that with an increasing Rac concentration, the reduction peak current increases. The above results indicate that there must be some relationship between the Rac concentration and the reduction current, which maybe a potential application of CDs@Au in the construction of electrochemical Rac sensors [16].

3.4 Electrochemical determination of Rac

The electrochemical measurement of RAC was achieved by differential pulse voltammetry (DPV) by the following steps (Figure 6) [22]. First, Rac was concentrated in advance, and the potential was set to 0.85V for 30s [26]. Then, the DPV response was recorded. The relationship of the curve between the DPV peak current and the Rac concentration has a good linear correlation coefficient of 0.9999 in a range of 0.01 to 32.5 mg/L, furthermore, the detection limit is 1.2 μg/L, which is below the value (1.5 μg/L) reported in [15].Table 1 summarizes the comparison of CDs@Au/GCE with other modified electrodes for the electrochemical measurement of Rac [23]. Compared with some of the previously reported electrochemical detection methods for Rac, our proposed sensor has a significantly improved sensor performance as demonstrated in this work. Compared with traditional electrodes, our sensors have many advantages and can be used for on-site inspections.

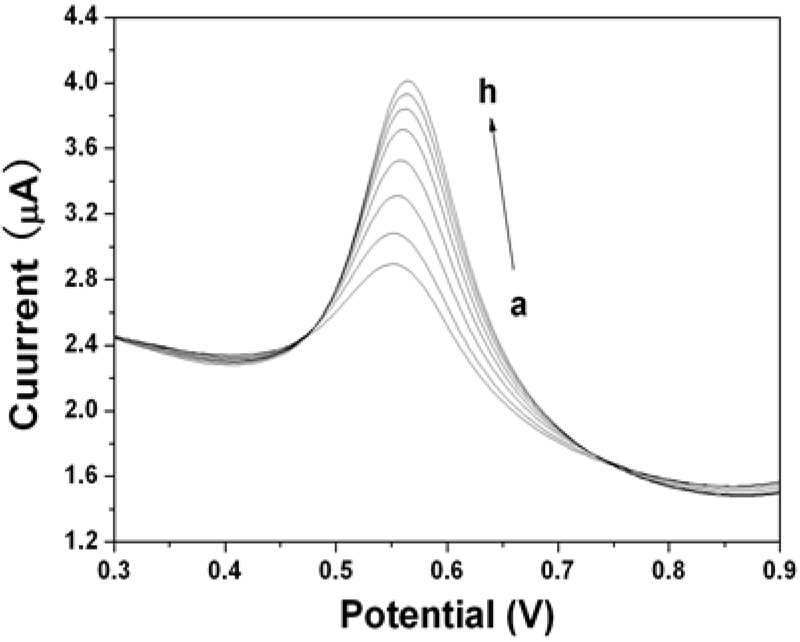

Figure 6. DPV of CDs@Au/GCE in a 0.2 mol/L PBS (pH 7.0) solution with 0, 0.10, 0.22, 0.34, 0.44, 0.51, 0.56, and 0.60 mg/L Rac (from a to h, respectively).

Table 1. Comparison of eletrodes modified with different materials for Rac detection

Modified electrode	LOD (nM)	Linear range (μM)	Ref.
GO/GCE	50	0.075-3.0	[23]
MWCNT-MIM/SPE	6	0.02-2.0	[24]
MWCNT/GCE	59	0.15-6.0	[25]
OMC/GCE	60	0.085-8.0	[26]
Au/OMC/GCE	4.4	0.03-74.0	[16]
MCF/CPE	10	0.05-3.3	[27]
CDs@Au/GCE	3.5 (1.2 μg/L)	0.03-96 (0.01- 32.5 mg/L)	This work

In this paper, the repeatability and stability parameters of the sensor are studied by an amperometric method[26]. By measuring the response current of the sensor to the 0.2 mg/L Rac solution on CDs@Au/GCE, the repeatability of the sensor was observed. After 10 parallel experiments, the sensordemonstrates good repeatability, and the relative standard deviation (RSD) is 2.19%. The stability of CDs@Au/GCE was tested for one month. The sensors were stored in a refrigerator at 4 °C and tested each day over a period of 10 days, and then for every 5 days over the next 20 days. For convenience, the average current response (n = 3) was set to 100% on the first day. The current response of 0.2mg/L RaconCDs@Au/GCE retains 91.0% of the original value after 1 month, indicating that the sensor has good stability.

3.5 Electrochemical detection of Racin real samples

Using an ultrasonic crushing method, 10 mL of pork (10 g) solution was prepared to estimate the consistency and potential applications of the present sensor [22]. Considering that the estimated Rac content value was very low and could be seen as errors, 0.10 mg/L Rac was added into the pork solution for the recovery tests. The results show that the average recovery of Racis 96.7–102%,the above results show that the sensor exhibits satisfactory results for Rac determination and that the applied method provides good accuracy for sample detection.

4. CONCLUSIONS

CD-reduced gold nanoparticles were used to develop electrochemical sensors for Rac determination. The kinetic characteristics of the improved sensor show that the reaction of the analyte on the electrode surface is controlled by diffusion. The results show that the sensor has good electrocatalytic ability ofRac, which leads to an increase in the peak current. Due to its high sensitivity and, good reproducibility and stability, as-prepared sensor material shows application potential for Racanalysis.

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