

# Electrochemical determination of clenbuterol with differential pulse voltammetry technique at carbon dots-based rice straw-modified electrode

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**Abstract.** In the present study, the carbon dots (CD) were extracted from rice straw (RS) and denoted as RSCD. The obtained material was examined by using techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), and nitrogen adsorption/desorption isotherms. The RSCD material was employed as an electrode modifier to improve the effectiveness of an electrochemical sensor for determining clenbuterol (CLB). RSCD showed fabulous oxidation towards clenbuterol. The modest detection limit could confirm an LOD value of 0.094  $\mu\text{M}$  within a linear range between 0.1 and 1.8  $\mu\text{M}$  under suitable conditions and the proposed technique — the differential pulse voltammetry (DPV). The developed sensor exhibited several benefits: simplicity, low cost, effortless generation, fabulous selectivity, and good reproducibility.

**Keywords:** carbon dots-based rice straw, clenbuterol determination, electrochemical sensor, DPV

## 1 Introduction

Clenbuterol (CLB), an exemplary  $\beta_2$ -adrenoreceptor agonist, has primarily been employed in treating asthmatic bronchitis [1]. Because of its ability to enhance protein deposition and lipid degradation, CLB has been illicitly introduced into livestock feed to cut costs [2]. However, a prolonged use of CLB in high doses as a feed additive can result in its accumulation in animal tissues, leading to cardiovascular and central nervous system disorders [3]. Consequently, the use of  $\beta$ -agonists as growth promoters for livestock has been prohibited in certain regions, such as Europe (Commission of the European Communities Council Directive 96/23/EC, 1996) and China (The Announcement Ministry of Agriculture) [4]. The

meticulous screening of CLB traces has become crucial for advancing stock breeding and safeguarding public health.

Until present, diverse analytical techniques have been documented for determining CLB, encompassing gas chromatography-mass spectrometry (GC-MS) [5], liquid chromatography-MS (LC-MS) [6], high-performance LC (HPLC) [7], capillary electrophoresis (CE) [8], and enzyme-linked immunosorbent assay (denoted as ELISA) [9]. While these methods show promise due to their high selectivity and sensitivity, they come with inherent drawbacks such as high cost, sample pretreatment requirements, and complex, time-consuming operational procedures. Regarding CLB's molecular structure, it is evident that it

should exhibit electrochemical activity thanks to its phenolic hydroxyl group, which can undergo oxidation on the electrode surface. Consequently, electrochemical methods have gained attention for CLB detection, owing to their high sensitivity, portability, low cost, and rapid analysis. However, direct electrochemical detection of CLB remains limited. Previous studies have explored various approaches, including the Nafion (Nf)-Au colloid-modified glassy carbon electrode (GCE) [10], Pt nanoparticles-modified gold electrode [11], molecularly imprinted polymer (MIP)-modified solid binding matrix composite electrode (SBMCE) [12], Nf-modified carbon-paste electrode [13], poly(acid chrome blue K)/graphene oxide-Nf/GCE [14], and acetylene black (AB)-dihexadecyl hydrogen phosphate (DHP) composite film-modified GCE [15] for direct electrochemical CLB detection. Therefore, developing novel electrochemical methods for CLB determination is of significant importance and interest.

Carbon dots (CDs) serve as a compelling foundation for sensors because of their special features, including water solubility, minimal toxicity, elevated emission intensity, and enduring chemical stability over time [16]. The structure of CDs comprises a carbon core and a surface passivation layer containing functional groups, predominantly hydroxyl, carboxyl, or amine groups. These functional groups facilitate conjugation with other molecules, such as proteins [17]. In addition, rice straw, along with various biomass options, represents an extensive carbon reservoir suitable for the generation of carbon dots [18, 19]. While rice straw has been utilized as a food source for livestock [20, 21], it is predominantly burned in nearly all developing nations [22–24]. This widespread practice poses significant challenges to our living environments, contributing to issues such as elevated CO<sub>2</sub> and dust emissions. The disposal of rice straw, a by-

product of rice cultivation, is a global concern, leading to environmental and health problems worldwide.

As far as our understanding goes, the investigation of CLB-based on the RSCD-modified electrochemical sensor is still limited. In this work, the formation of an RSCD-based developed electrode was reported and utilized to determine clenbuterol with the DPV technique. The proposed method is effortless, cost-saving, and accurate. In addition, the resulting improved electrode is selective, reproducible, and fast.

## 2 Materials, apparatus and preparation of RSCDs

### 2.1 Materials

All Merck (Germany) analytical reagents (clenbuterol, Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>, NaOH, HNO<sub>3</sub>) were employed in the experiments. Solutions were prepared with double-distilled water. Glassware was cleaned by immersing it in a 1:2 HNO<sub>3</sub> solution for 4–6 hours and then rinsing it with distilled water before use.

### 2.2 Apparatus

Powder X-ray diffraction pattern (XRD) of RSCD was recorded with a D8 Advance Bruker diffractometer paired with a Cu-K $\alpha$  radiation source ( $\lambda = 1.5406 \text{ \AA}$ ). A scanning electron microscope (SEM; Hitachi S-4800) was applied to detect the surface morphologies of the synthesized materials. Nitrogen adsorption/desorption isotherms were recorded on a Micromeritics-ASAP 2020 machine; and the material was degassed at 120 °C for 5 h. Furthermore, the specific surface area was determined by using the Brunauer-Emmett-Teller (BET) formula, and the mesoporous surface area was calculated with the *t*-plot technique. Voltammetric examinations were carried out with

a computerized polarography analyzer machine named CPA-HH5 (Vietnam), in which a conventional electrode system (consisting of Ag/AgCl/saturated KCl as the reference electrode, a Pt wire as the auxiliary electrode, and a bare GCE (2.8 mm in diameter) or RSCD-modified electrode as the working electrode) was used for all electrochemical experiments.

### 2.3 The preparation of RSCD

Carbon quantum dots (RSCDs) were synthesized via a hydrothermal route. Rice straw underwent iterative washing with distilled water, subsequent desiccation at 80 °C, and meticulous fragmentation. Following this, one gram of the finely fragmented rice straw was dispersed in 50 mL of distilled water and subjected to agitation for 15 minutes prior to its transference into a 100 mL sample digestion vessel made of stainless steel. The hydrothermal synthesis procedure was executed in a drying oven set at 180 °C for 24 hours. Subsequent to the reaction, the mixture was allowed to undergo natural cooling to ambient temperature. The resultant solution, displaying a light yellow hue, was then transferred into a 15 mL centrifuge tube and subjected to centrifugation at a rate of 4000 revolutions per minute for 15 minutes to effect solid phase removal, thereby yielding the carbon quantum dots solution (RSCDs)

### 2.4 Preparation of RSCD/GCE

The unmodified electrode used in the study was a bare GCE with a diameter of  $2.80 \pm 0.10$  mm. This electrode underwent a series of preparation steps: it was polished with  $\text{Al}_2\text{O}_3$  powder (with a particle size of 0.050  $\mu\text{m}$ ), immersed in a 2.0 M  $\text{HNO}_3$  solution, cleaned with  $\text{C}_2\text{H}_5\text{OH}$  and deionized water, and left to air-dry at ambient temperature. The modification of the glass-carbon electrode was carried out as follows: RSCD ( $2.0 \text{ mg}\cdot\text{mL}^{-1}$ ) was dispersed under ultrasonic

irradiation for 65 minutes. Afterwards, 2.50  $\mu\text{L}$  of the resulting suspension was transferred onto the surface of the GCE, and the solvent was allowed to evaporate at ambient temperature for 5–6 minutes. The resulting RSCD-modified GCE was denoted as RSCD/GCE and stored in a desiccator as the as-prepared RSCD electrode.

## 3 Results and discussion

### 3.1 Characterizations

#### X-ray diffraction

The crystallinity diffractogram of the rice straw-based carbon dots was obtained with powder X-ray diffraction (XRD), as described in Fig. 1. This pattern exhibits two defined peaks at  $2\theta = 25.21^\circ$  and  $42.22^\circ$ , which confirms the unstructured nature-original of RSCD [25, 26]. The peaks correspond to the crystal lattice (002) and (101), which is the primary property of nanomaterials-based carbon [27]. The presence of O- and N-containing groups on the CDs is also an evidence for the amorphous origin of CDs [28].

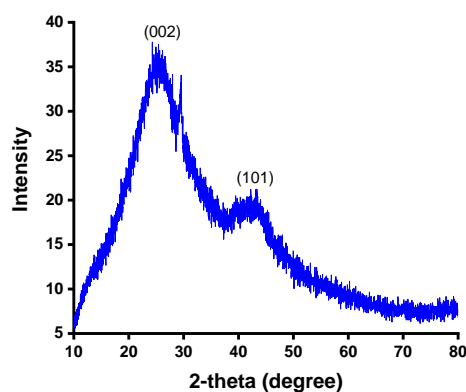


Fig. 1. XRD pattern of RSCD

#### Scanning electron microscopy

The morphology of RSCD was examined by using SEM and described in Fig. 2. As can be seen in the figure, the resulting RSCD exhibits a sponge-like porous structure with several sizes. This confirms that RSCD was successfully synthesized.

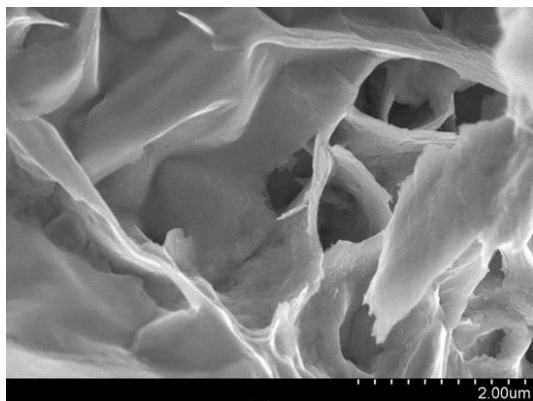


Fig. 2. SEM image of RSCD

### Nitrogen adsorption/desorption isotherms of RSCD

The nitrogen adsorption/desorption isotherms of the RSCD sample are illustrated in Fig. 3. As described in the figure, the IV-type (IUPAC) isotherm paired with the H4 hysteresis loop would be detected for RSCD, showing its porous property with the proof from micro pores. In addition, as a micro-sized porous material, this product has a large surface area of  $613.57 \text{ m}^2 \cdot \text{g}^{-1}$ .

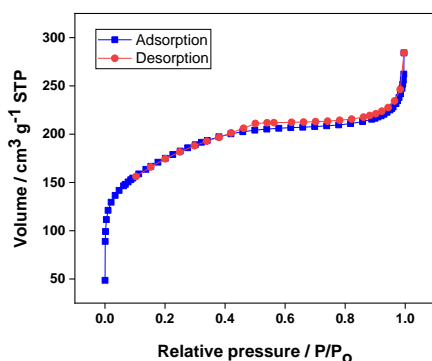


Fig. 3. Nitrogen adsorption/desorption isotherms of RSCD

## 3.2 Electrochemical examination of CLB

### Electrochemical behaviour

The electrochemical behaviour of different electrodes was examined by using the DPV technique (Fig. 4). In the figure, some employed electrodes contain the bare GCE and GCE

modified with RSCD. As can be seen in Fig. 4, no peak would be detected when employing two electrodes: bare GCE with CLB and RSCD-modified GCE without CLB. In contrast, a defined peak at 1.51 V would be found with the GCE modified with RSCD in the presence of CLB. Therefore, RSCD/GCE is a suitable electrode for detecting CLB in the next experiments.

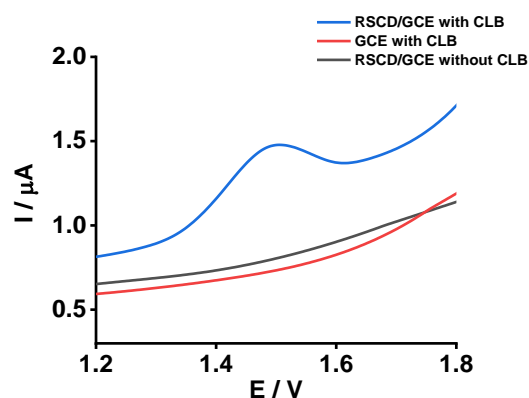


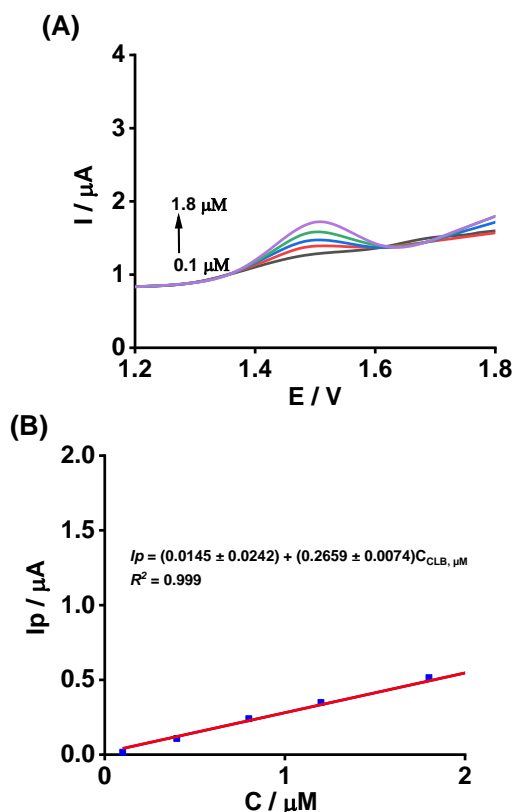
Fig. 4. DPV curves on bare GCE and RSCD/GCE modified electrode. Experimental conditions:  $10 \mu\text{M}$  clenbuterol;  $0.2 \text{ M}$  PBS buffer solution; potential scan range from 1.2 to 1.8 V;  $\text{pH} = 7.0$ ; scan rate of  $0.02 \text{ V/s}$

### Limit of detection (LOD)

The differential pulse voltammetric technique was applied to examine the electrocatalytic reaction of the modified electrode – RSCD/GCE – towards CLB's responses (Fig. 5A). The DPV curves were recorded with the adding concentration of CLB from 0.1 to  $1.8 \mu\text{M}$  in  $0.2 \text{ M}$  PBS ( $\text{pH} = 7.0$ ). It could be seen that the anodic peak current grows gradually when adding an orderly CLB concentration using RSCD-modified GCE (Fig. 5B). The close relationship between the oxidation peak current and the CLB contents is observed, and an obtained regression equation with the scope of  $0.1\text{--}1.8 \mu\text{M}$  and the LOD value of  $0.094 \mu\text{M}$  is expressed as follows:

$$I_p = (0.0145 \pm 0.0242) + (0.2659 \pm 0.0074) \times C_{\text{CLB}, \mu\text{M}}$$

$$R^2 = 0.999$$



**Fig. 5.** (A) Differential pulse voltammetric responses of CLB at RSCD/GCE with concentration scope of 0.1–1.8  $\mu\text{M}$ ; (B) Relationship between  $C_{\text{CLB}}$  ( $\mu\text{M}$ ) and  $I_p$  ( $\mu\text{A}$ ). Experimental conditions: 0.2 M PBS buffer solution; potential scan range from 1.2 to 1.8 V; pH = 7.0; scan rate of 0.02 V/s

Furthermore, the modified electrode – RSCD/GCE – exhibits a good sensitivity of  $0.2659 \pm 0.0074 \mu\text{A } \mu\text{M}^{-1}\cdot\text{cm}^{-2}$ . A great determination ability of the suggested electrochemical sensor towards CLB signal is evidenced from the low LOD of 0.094  $\mu\text{M}$  ( $3\sigma$  method). Approximately, the analysis benefit of the modified electrode is confirmed by the good sensitivity and LOD value of the sensor, compared with that published in previous reports (Table 1).

Table 1 provides information on the LOD, LOQ value and linear range at various employed electrodes for detecting CLB. Compared with other sensors, as can be seen from the table, the determination outcome of the RSCD/GCE sensor in this work is superior to that of the majority of previous reports.

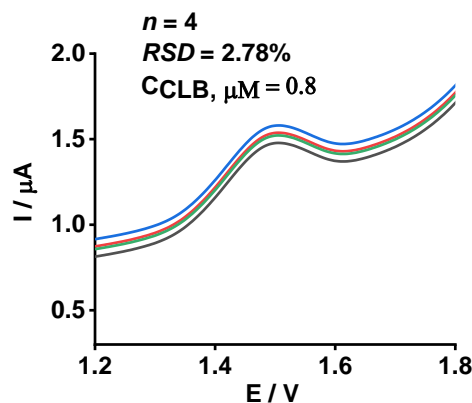
**Table 1.** Comparison between proposed modified electrode and previously detected CLB articles

Employed electrode	Linear range	LOD	LOQ	Ref.
BDD	0.50–50.0	0.31	1.085	[29]
SGS/MWCNT s-COOH/GCE	0.01–5.0	0.0050	0.0175	[30]
AuNPs/GCE	0.01–0.20	0.0081	0.0283	[31]
Pd/BDD	1.6–637.7	0.604	2.114	[32]
RSCD/GCE	0.10–1.80	0.094	0.282	This work

Note: BDD: boron-doped diamond; SGS: sulfonated graphene sheets; MWCNTs: multi-walled carbon nanotubes; AuNPs: gold nanoparticles; GCE: glassy carbon electrode.

### Reproducibility experiments

Reproducibility is a crucial factor for an electrochemical electrode, paving its practical property in *in-situ* application. The DPV technique was employed to examine the reproducibility of the electrodes.



**Fig. 6.** DPVs of CLB at 4 times of modifying GCE with RSCD. Experimental conditions: 0.8  $\mu\text{M}$  clenbuterol; 0.2 M PBS buffer solution; potential scan range from 1.2 to 1.8 V; pH = 7.0; scan rate of 0.02 V/s

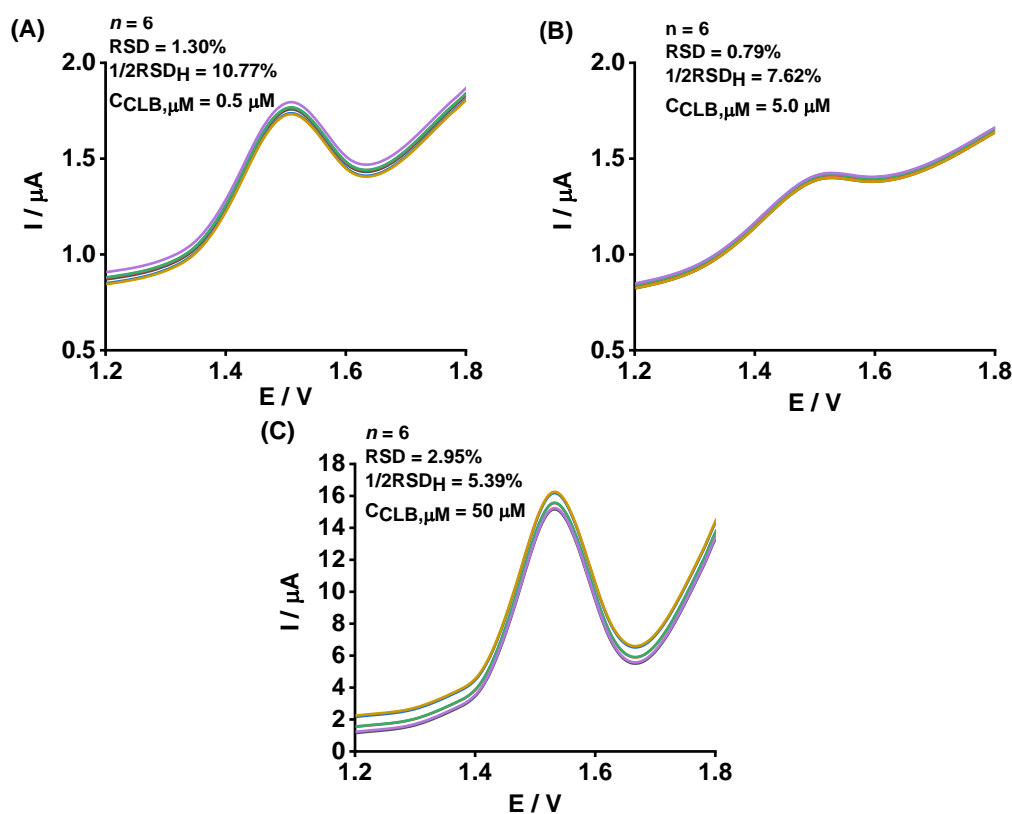
The reproducibility was investigated by assessing a 0.8  $\mu\text{M}$  CLB solution in 0.2 M PBS (pH = 7.0). The test consisted of recording DPV curves of CLB by using four RSCD/GCEs (meaning four times modifying RSCD onto one certain bare GCE). The test confirmed the good reproducibility

of the RSCD-based electrodes with a small RSD value of 2.78%; this value is not greater than 5%. Therefore, the obtained outcome strongly suggests the great reproducibility of the RSCD-modified electrodes for examining CLB.

### Repeatability of DPVs

The repeatability of the proposed electrochemical technique was investigated via six consecutive measurements at varying concentrations of CLB: 0.5, 5.0, and 50.0  $\mu\text{M}$ . The resultant relative

standard deviations were 1.30, 0.79, and 2.95%, respectively; all of which fall below the anticipated threshold of  $1/2 \times \text{RSD}_{\text{Horwitz}}$  (determined as 10.77, 7.62, and 5.39%, respectively) [28]. Consequently, these commendable RSD values across successive measurements suggest that the RSCD/GCE configuration can be reliably employed for the detection of CLB at both low and high concentrations.



**Fig. 7.** DPV curves of several concentrations of CLB with RSCD/GCE ( $n = 6$ ): (A) 0.5  $\mu\text{M}$ ; (B) 5.0  $\mu\text{M}$ ; (C) 50  $\mu\text{M}$ . Experimental conditions: 0.2 M PBS buffer solution; potential scan range from 1.2 V to 1.8 V; pH = 7.0; scan rate of 0.02 V/s

## 4 Conclusion

In this work, the RSCD-modified GCE was improved and employed to detect clenbuterol with the DPV technique. RSCD/GCE is a fabulous modified electrode for detecting CLB. The

proposed method is easy, fast, economical and favorable for determining clenbuterol pharmaceuticals.

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## Conflict of interests

The authors declare no conflict of interest regarding the publication of this article.

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