Hue University Journal of Science: Natural Science
Vol. 131, No. 1D, 89–96, 2022

pISSN 1859-1388
eISSN 2615-9678

Determination of clenbuterol with differential pulse anodic stripping voltammetry technique at tungsten disulfide-modified electrode

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(*Received: 26 February 2022; Accepted: 27 April 2022*)

Abstract. In the present work, tungsten disulfide was synthesised and characterised by using X-ray diffraction and scanning electron microscopy. This material was employed to modify a glassy-carbon electrode to analyse clenbuterol. The results show that tungsten disulfide displays electrocatalytic activity toward clenbuterol oxidation. The proposed technique is prospective for clenbuterol examination. The limit of detection and the linear range are 0.51 µM and 1–210 µM.

Keywords: tungsten disulfide, clenbuterol, electrochemical analysis

1 Introduction

Along with the strong development of industrialisation and modernisation in Vietnam, toxic and dangerous chemicals are used in large quantities and types. The use and abuse of antibiotics, chemicals, and chemicals banned in livestock production are alarming. One of the most strictly forbidden substances is the weight-stimulating hormone known as clenbuterol (CLE), a synthetic chemical compound of phenylethanolamine, belonging to the family of β-agonist prohibited for use in livestock worldwide [1]. Numerous studies have indicated that using hormones in livestock production leads to hormone residues in animal products. These residues, in turn, disturb the balance of human hormones when consumed and cause harm to the environment. When the hormone is released and assimilated by other organisms, it can also cause reproductive and growth disorders [2–4]. The residue of growth hormone in food is the cause of abnormal changes in the body's development, causing genetic changes and cancer and stimulating tumours to grow faster [5]. Therefore, controlling and assessing antibiotic abuse in cattle raising is a concern not only for developed countries but also for developing countries like Vietnam. Consequently, it is required to create perfect analytical methods with high sensitivity, selectivity and low detection limit to identify these organic compounds accurately. Numerous multi-function analytical methods have been developed and widely applied, such as molecular absorption, high-performance liquid chromatography, gas chromatography coupled mass spectrometry, and electrochemical analyses. However, these techniques have many limitations, like the high equipment and analysis costs. Meanwhile, the electrochemical methods [6-8] are advantageous in terms of high sensitivity, accuracy, selectivity, and low detection limit. Particularly, these low costs make the methods very suitable for qualitative or direct *in-situ* quantification. Currently, researchers are paying much attention to developing working electrodes,

DOI: 10.26459/hueunijns.v131i1D.6716

especially those modified with nano-sized materials [9-11].

Clenbuterol (or $C_{12}H_{18}Cl_2N_2O$) is a kind of β_2-adrenergic agonist primarily used in the treatment of asthmatic bronchitis [12]. As it can increase protein deposition and lipid degradation, CLB has been abused in pig pannages as well [13]. However, because of its long half-life and slow metabolism in animal bodies, CLB can cause health disorders, such as muscular tremors, heartthrob, glaucoma, prostatitis, and even death in children and elderly people. Moreover, clenbuterol is stable and decomposes at temperatures over 172 °C. Therefore, the World Health Organisation has forbidden using clenbuterol in stock breeding.

Possessing unique physical and chemical properties, the nanomaterials synthesised based on transition metal dichalcogenides consisting of 2D layers have attracted much attention in recent years, especially in sensor applications. Tungsten disulfide (WS_2) comprises S-W-S interlayers with a triangular prismatic configuration. WS_2 nanomaterials are synthesised by separating or encapsulating precursor materials with the hydrothermal method for application in gas sensors and biosensors. This material is an excellent biosensor because of its distinctive biocompatibility, large active surface area, and high catalytic efficiency. Some of the latest reports have shown that WS_2 is a potential material worthy of research for wide application. Zhang et al. used a WS_2 electrode to determine copper adenine and guanine with a detection limit of 5×10^{-8} and 9×10^{-8} M (14). Yue et al. identified dopamine in human urine (uric acid) with a limit of detection (LOD) of 0.01 μM [15]. Blanco et al. identified ponceau 4R and tartrazine with a LOD of 7.8×10^{-6} and 4.5×10^{-6} M (16).

Based on the arguments above, we developed a von-ampere method using WS_2-modified glassy-carbon electrodes (WS_2/GCE) to identify clenbuterol in animal products. The technique is highly feasible in laboratories equipped with multifunctional electrochemical analysis equipment. This paper presents the results of using a WS_2/GCE electrode to quantify clenbuterol with the differential pulse anodic dissolution von-ampere method (DP-ASV).

2 Experimental

2.1 Material and apparatus

All chemicals used were analytical reagents of Merck (Germany). Double-distilled water was used to prepare the solutions. Glassware was washed by soaking in a 1:2 HNO_3 solution for 4–6 hours and rinsing with distilled water before use.

Powder X-ray diffraction patterns of WS_2 were recorded on a D8 Advance Bruker diffractometer equipped with a Cu-Kα radiation source (k = 1.5406 Å). A scanning electron microscope (Hitachi S–4800) was employed to examine the surface morphologies of the as-prepared materials. Voltammetric measurements were performed on a CPA-HH5 computerised polarography analyzer (Vietnam) with a conventional three-electrode system (Ag/AgCl/saturated KCl as the reference electrode, a platinum wire as the auxiliary electrode, and a bare GCE (2.8 mm in diameter) or WS_2-modified GCE as the working electrode).

2.2 Preparation of electrode

The bare electrode was a glass-carbon electrode with a diameter of 2.8 ± 0.1 mm. The electrode was polished with Al_2O_3 powder (particle size of 0.05 μm), soaked in a 2 M HNO_3 solution, washed with ethanol and distilled water, and dried naturally at ambient temperature. The modification of the GCE was carried out as follows: WS_2 (2 mg·mL^{-1}) was dispersed under ultrasonic irradiation for 1 h. Subsequently, 2.5 μL of the suspension was dripped onto the GCE surface, and the solvent was allowed to evaporate at ambient temperature for 2–3 min. The obtained WS_2-modified GCE was denoted as WS_2/GCE and stored in a desiccator (as-prepared WS_2 electrode).

3 Results and discussion

3.1 Material characterisation

X-ray diffraction

The crystal structure of the WS_2 material is presented in Fig. 1. The peaks were found at 2θ (*hkl*) of 14.39° (002), 23.09° (222), 28.15° (100), 30.22° (101), 34.8° (102), 38.32° (103), 44.94° (006), 50.61° (105), 55.4° (106), 59.4° (110) and 65.27° (114) (based on JCPDS no: 87-2417 and 84-1398).

Scanning electron microscopy

Scanning electron microscopy was employed to verify the morphology of WS_2 nanostructures. Fig. 2 describes the sheet-like structure of WS_2 nanostructures. This confirms that WS_2 was successfully synthesised.

3.2 Electrochemical response

Electrochemical behaviours of different electrodes

The DPV technique was employed to examine the electrochemical behaviours of CLE at various electrodes. The voltage of the DPV was between –0.2 and 1 V. As can be seen in Fig. 3, no peaks appear for the bare GCE, while a well-defined peak of CLE is observed for WS_2/GCE at 0.72 V. This peak indicates that WS_2/GCE is prospective to detect CLE.

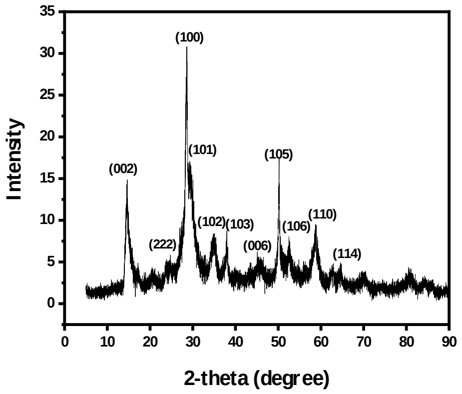

Fig. 1. XRD diffractogram of WS_2

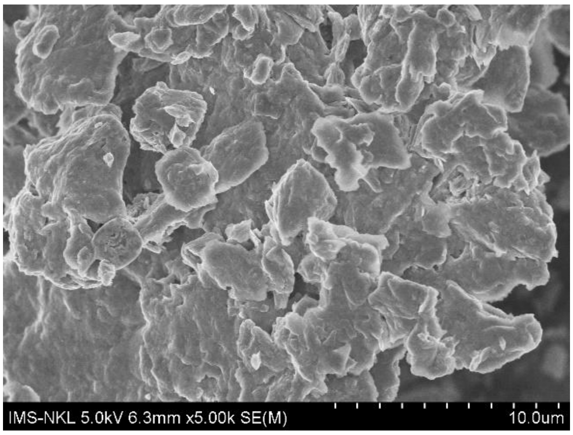

Fig. 2. Scanning electron microscopic image of WS_2

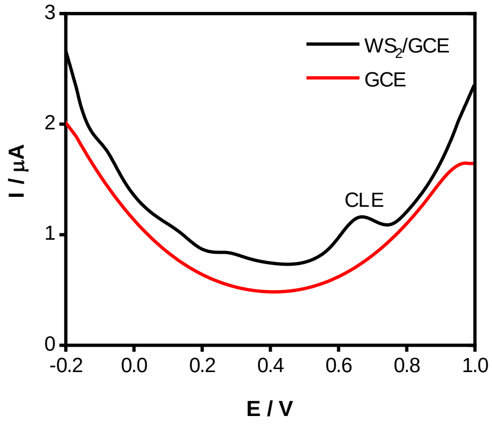

Fig. 3. DPV curves of clenbuterol at GCE and WS_2/GCE with 5 μM clenbuterol in 0.1 M PBS (pH 7)

Effect of pH

The influence of pH (3–8) on voltammetric responses of CLE was examined in the PBS buffer solution (Fig. 4A). It is clear from Fig. 4B that the intensity of I_P for CLE increases with the pH between 3 and 5 and reaches the highest point at pH 5. Beyond this pH, the intensity decreases. As a result, pH 5 was chosen for the subsequent measurements.

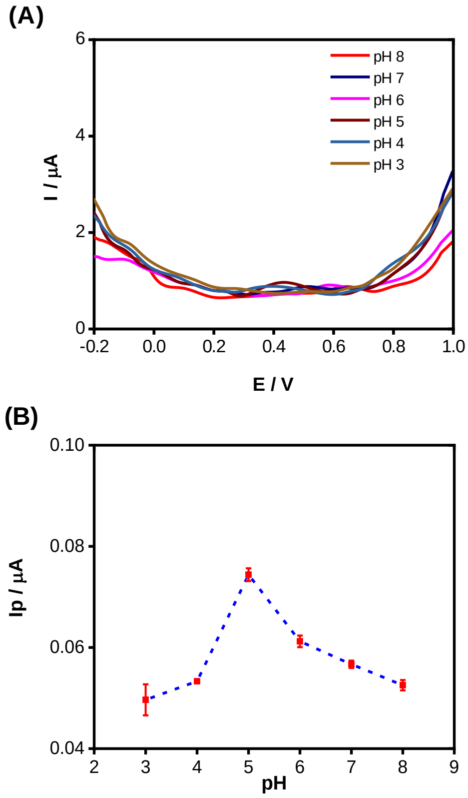

Fig. 4. (A) DPV curves of clenbuterol at several pHs; (B) Relationship between I_P and pH; 5 μM of clenbuterol in 0.1 M PBS

3.3 Optimising working parameters

Accumulation potential

The DP-ASV technique was utilised to examine the accumulation potential (E_{cc}) for the CLE signal. The accumulation potential was measured from –0.4 to 0.4 V. Fig. 5 reveals that the peak current of the DP-ASV increases with the accumulation potential up to –0.1 V, reaching the highest point and then gradually decreasing afterwards. Therefore, –0.1 V could be seen as the optimised accumulation potential for the CLE signal in the subsequent measurements.

Accumulation time

The accumulation time (t_{cc}) was measured from 0 to 120 s. Fig. 6 shows that the peak current of the DP-ASV increases with the accumulation time up to 60 s, reaching the highest point and then remaining stable. Therefore, to save time, 60 s could be seen as the optimised accumulation time for the CLE signal in the following experiments.

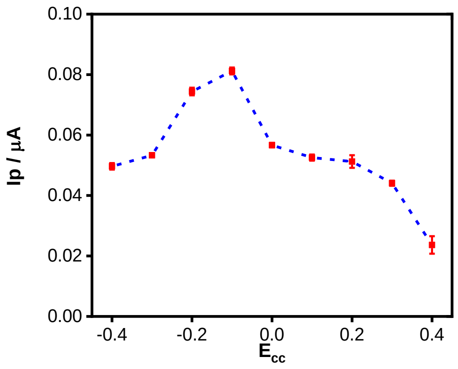

Fig. 5. Linear plot of I_P versus E_{cc}; 5 μM of clenbuterol in 0.1 M PBS (pH 5); Accumulation time 15 s

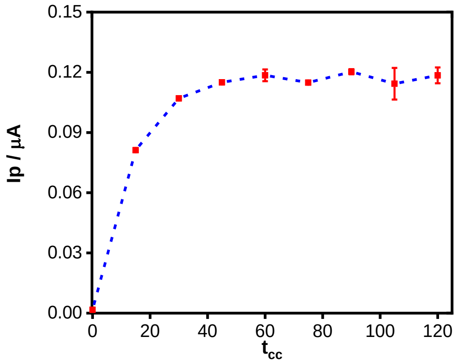

Fig. 6. Relationship between I_P and t_{cc}; 5 μM of clenbuterol in 0.1 M PBS (pH 5); Accumulation potential –0.1 V

Effect of interferents

Relative error (RE) is used to examine the chemical interference during the examination of an analyte. If the absolute value of RE is less than five per cent, interferents do not affect the measurements and vice versa [17, 18]. Organic interferents commonly considered in medicines and biological fluids include paracetamol, uric acid, ascorbic acid, and caffeine. The analyte solution might contain inorganic salts such as Na_2SO_4, $Ca(NO_3)_2$, $BaCl_2$, and $(NH_4)_2SO_4$. It is necessary to study the interference by progressively increasing the interferent concentration in a 0.1 M PBS buffer (pH 5) containing 5 μM CLE. Table 1 indicates that at very high concentrations — more than 16-fold for the organic and 160-fold for inorganic interferents), none of these substances interferes with the examination of CLE at the modified electrodes. Therefore, the suggested DP-ASV approach displays good selectivity for determining CLE.

Table 1. Influence of interferents on oxidation peak current, 5 μM of clenbuterol in 0.1 M PBS (pH 5)

Interferents	**Concentration (μM)**	**RE (%)**
Na_2SO_4	250	–3.84
$BaCl_2$	220	2.88
$Ca(NO_3)_2$	300	–4.47
$(NH_4)_2SO_4$	180	0.31
Paracetamol	80	–0.33
Uric acid	120	4.59
Caffeine	180	–3.28
Ascorbic acid	80	–3.73

Repeatability

The electrode repeatability was studied at two CLE concentrations: 10 and 200 μM in the 0.1 M PBS solution and pH 5. The peak current does not change significantly after ten repeated measurements with these solutions (Fig. 7). The calculated RSD for the 10 and 200 μM CLE solutions is 4.08 and 2.1%. Additionally, the Horwitz function ($RSD_H = 2^{1-0.5\times\log C}$, where C is the analyte concentration in decimal fractions) was utilised to evaluate the proposed technique. The RSD values are less than ½ × RSD_H, indicating that the method is highly repeatable [19].

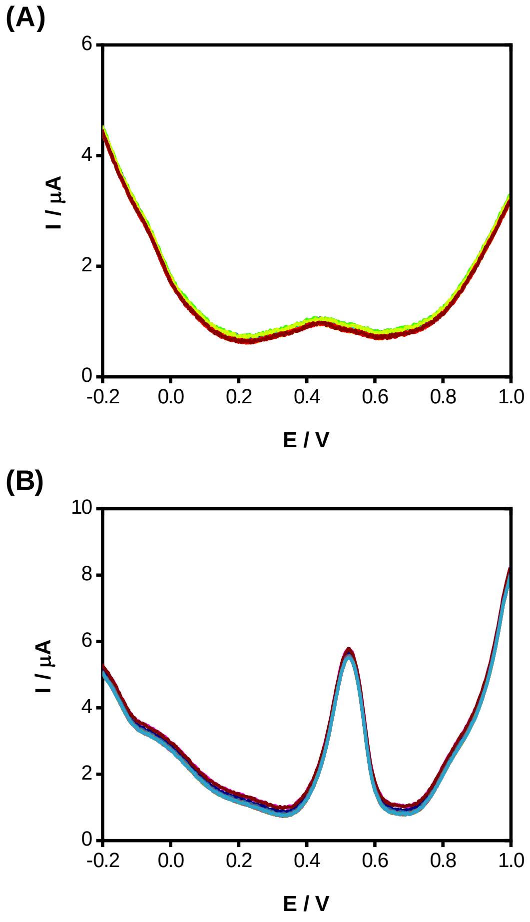

Fig. 7. DP-ASV curves for clenbuterol with different concentrations: (A) 10 μM; (B) 200 μM in 0.1 M PBS buffer (pH 5)

Linear range, detection limit and quantitative limit

In this section, the CLE concentration was from one to 210 μM, and the DP-ASV curves are presented in Fig. 8A. Fig. 8B shows the corresponding calibration line with the following linear equation.

$I_{p,PAR}$ (μA) = (0.02 ± 0.02) + (0.022 ± 0.003) × C_{CLE} (μM); $R^2 = 0.999$

The calculated LOD for CLE is 0.51 μM (taken as 3 σ/*S*, where *S* is the sensitivity result and σ is the standard deviation result of the black signal), and the quantitative limit is 1.53 μM.

For the examination of CLE, this electrode has a linear value range and LOD that is quite favourable for those recorded in the references (Table 2).

It is proven that the DP-ASV technique employing WS_2/GCE displays the potential to apply to analyse clenbuterol in practical samples.

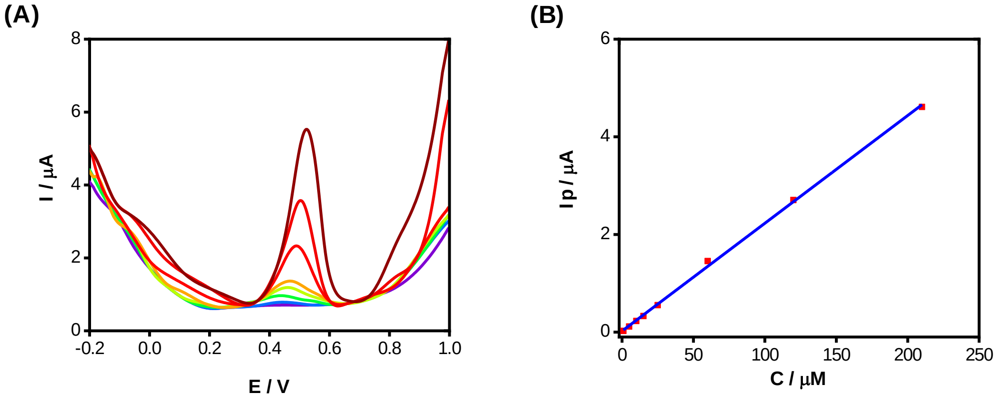

Fig. 8. (A) DP-ASVs of clenbuterol with rising concentrations: 1, 5, 10, 15, 25, 60, 120, 210 μM; (B) Plot of I_p versus C_{CLE}

Table 2. Comparison between the LOD within the clenbuterol electrochemical examination with previous reports

No.	Electrode	Technique	Linear range (μM)	LOD (μM)	Ref.
1	PtNPs	DP-ASV	0.1 – 0.8	0.044	[20]
2	MWCNT-film	DP-ASV	0.0025 – 3.2	0.0010	[21]
3	Nafion-Au colloids/GCE	DP-ASV	8 – 100	1	[22]
4	MWNTs/SPCE	Amperometric	0.00096 – 0.32	0.00024	[23]
5	GO/GCE	DP-ASV	0.08 – 3.2	0.048	[24]
6	WS_2/GCE	DP-ASV	1 – 210	0.51	This work

4 Conclusion

In this study, the WS_2-modified glassy-carbon electrode was developed and utilised to determine clenbuterol with the DP-ASV method. WS_2 is an excellent electrode modifier for clenbuterol determination. This proposed method is simple, fast, cost-saving, and suitable for analysing clenbuterol in livestock products.

Funding statement

This work was supported by the Master and PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), Vingroup Big Data Institute (VinBigdata), under code VINIF.2021.ThS.96

Conflict of interests

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

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