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Fabrication of an electrochemical sensor based on a graphene/Au composite for the determination of clenbuterol in beef samples

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To achieve fast clenbuterol determination, a super-sensitive amperometric biosensor was fabricated on the basis of Au nanoparticle-modified graphene oxide nanosheets (GR/Au). Antigen determination was achieved by Au measurement via positive differential pulse voltammetry (DPV) in KCl solution, and the GR/Au–clenbuterol nanocomposite capture occurred on the immunosensor via competitive immunoreactions. The results displayed a linear response (0.01–15.0 ng/mL) with a detection limit (DL) as low as 7.6 pg/mL. Furthermore, clenbuterol detection in real beef specimens could be desirably addressed by the remarkably stable and specific electrochemical immunoassay proposed herein.

Keywords: Clenbuterol; Electrochemical sensor; Graphene; Gold nanoparticles; Glassy carbon electrode; Beef

1. INTRODUCTION

Phenyl ethanolamines containing diverse substituents on the aromatic ring and a terminal amino group are termed β-agonists and include compounds such as salbutamol, zilpaterol, cimaterol, ractopamine and clenbuterol. Carcass fat reduction and growth rate enhancement could be achieved by β-agonists in poultry [1], pig [2, 3], and calf [4] feeds by functioning as repartitioning agents, as indicated in many studies concerning the livestock industry. To the best of our knowledge, the most efficacious β-agonist for enhancing growth is clenbuterol [5]. Nevertheless, acute poisoning symptoms may be induced in humans by aggregated clenbuterol residuals in animal tissues [6]. In studies on

separate events having occurred in various countries, researchers presented symptoms resulting from clenbuterol residue-induced food poisoning [7, 8]. Furthermore, tremendous economic loss and influence on edible animal product exportation might be caused by drug residuals. Hence, a majority of countries have imposed bans on clenbuterol utilization.

To check whether illegal drugs exist in animal excreta and tissues and further combat the illegal adoption of β-agonists and their related compounds, regulatory institutions have exerted great efforts to test these excreta and tissues [9]. For instance, with respect to clenbuterol detection in animal tissues and feeds, diverse analytical techniques have been used, including liquid chromatography featuring electrochemical/mass spectrometric determination [10, 11], gas chromatography featuring mass spectrometry [12, 13], enzyme-linked immunosorbent measurements featuring polyclonal/monoclonal antibodies [14, 15], capillary electrophoresis featuring amperometric determination [16], electrochemical techniques featuring differential-pulse voltammetry [17] and immunosensors featuring surface plasmon resonance [18, 19]. Specifically, due to their convenient use, rapid response, inexpensiveness, simplicity, desirable sensitivity and reliability, electrochemical techniques have been popularly employed in analyte determination.

Recently, researchers have shown increasing interest in a newly emerging carbon-based nanomaterial—graphene (GR) [20, 21]. Featuring low cost, elevated surface area and desirable electrical conductivity [22-24], GR is regarded as a favorable electrochemical material. Certain biomolecule determination methods have used decorated GR electrodes [25-27]. Nevertheless, there is a tendency for graphite formation via π–π stacking and van der Waals interactions after GR restacking or the formation of irreversible agglomerates if GR sheets are not desirably separated from each other [28, 29]. Thus, non-covalent functionalizations or chemical modifications have been employed for GR solubility enhancement via diverse techniques [30-32]. Fields concerning chemical sensors, catalysis and energy storage have witnessed the potential adoption of GR-based hybrids with metallic nanoparticles in recent years. Thermal and electronic conductivity can be enhanced by GR-based hybrids with metallic composite materials [33, 34]. GR sheets accumulating impedance under dry conditions is another significant trait of metal nanoparticle-GR adhesion. With the distance between GR sheets extended by metal nanoparticle spacers, GR's two faces become accessible [35].

Featuring distinct optical surface traits, nano-Au has gained widespread use as a catalyst to promote diverse chemical reactions [36]. In terms of electrochemistry, a newly emerging functional material—a hybrid made up of nano-Au and GR—would potentially be adopted. To date, Au/GR hybrid fabrication has been achieved by two major techniques. In one of the techniques, graphene can be linked with nano-Au via diallydimethyl ammonium chloride (cationic polyelectrolyte poly) and other intermediaries, where Au-media-graphene hybrid formation is eventually achieved. Since the intermediaries between nano-Au and graphene are not conductive, they functioned as a barrier, thus leading to undesirable hybrid conductivity as a negative of this technique. This work presents nano-Au development (in situ) on graphene to offset this shortcoming. The uniform distribution of nano-Au on GR had been indicated by several researchers via this technique [37, 38].

Herein, a solution-based method was used to prepare nano-Au-decorated GR nanocomposites via a chemical co-reduction. The reduction of GO and the in situ deposition of nano-Au were achieved in a one-pot process. The as-prepared nanocomposites showed good dispersibility, and their catalytic

activities were evaluated by determination of clenbuterol. This modified electrode exhibited high sensitivity and stability in the detection of clenbuterol. We also studied the use of the GR/Au/GCE for sensing certain other biomolecules, including ractopamine, dobutamine, and salbutamol.

2. EXPERIMENTS

2.1. Chemicals

Shanghai Chemical Reagents Co. Ltd. was the material source for $HAuCl_4$. Nanjing Xianfeng NANO Materials Tech Co. Ltd. was the material source for graphite oxide (GO). Sigma-Aldrich Company (St Louis, MO) was the material source for salbutamol, the antibody of clenbuterol hydrochloride (CHanti), ractopamine and clenbuterol, all of which were used as received. Mixing NaH_2PO_4 (0.1 M) and Na_2HPO_4 (0.1 M) solutions allowed the creation of phosphate buffer solutions (PBS) at various pH values. All reagents were of analytical grade. All the experiments were performed using double-distilled water.

2.2. Synthesis of GR/Au nanocomposites

Suspending graphite oxide into water yielded a brown dispersion. The thorough removal of the remaining acids and salts in this dispersion could be achieved by submitting it to dialysis. This was followed by the dispersion of the above suspensions into water to generate the next dispersion (1 mg/mL). The graphite oxide dispersion was sonicated for 6 h to exfoliate graphite oxide to obtain GO. After sodium citrate was employed to chemically co-reduce GO and Au(III), the preparation of the Au/GR nanocomposites was achieved. To be brief, the mixture of $HAuCl_4$ solution (25 mL, 0.2 mg/mL) and the added as-prepared GO dispersion (4 mL) was stirred for 60 min to enhance the graphene surface–Au ions interaction. This was followed by the dropwise addition of sodium citrate (940 μL) to the aforementioned mixture. This final mixture was heated for 120 min at 80 °C. The product was separated by centrifugation, washed with ethanol and double-distilled water, and then vacuum dried at ambient temperature. Except for only reducing GO, an identical process was employed for GR preparation.

2.3. Preparation of modified electrode

The GCE was submitted to Al_2O_3 (0.5 and 0.05 μm) powder polishing, water rinsing, and sequential sonication in ethanol and double-distilled water. This was followed by blowing a gentle nitrogen stream over the as-cleaned GCE. GR/Au or GR DMF/aqueous solution (10 μL, 9:1 DMF/H_2O volume ratio) or nano-Au aqueous solution with 1.0 mg mL^{-1} as the GR/Au or GR concentration was placed onto the clean GCE followed by drying at ambient temperature. The products were referred to as GR/Au/GCE, GR/GCE and nano-Au/GCE, respectively. As-modified electrodes were subsequently immersed for 2 h in CH_{anti} solution, followed by Milli-Q water rinsing.

2.4. Characterizations

Voltammetric measurements and characterization of the electrodes were recorded on a CHI 770 electrochemical analyzer from Shanghai Chenhua Instrument Company (China). These studies used a traditional triple-electrode configuration composed of counter, reference and working electrodes, which were respectively filled by platinum, Ag/AgCl and an original or decorated glassy carbon electrode (GCE, with a diameter of 4 mm). Cyclic voltammetry was conducted in 10.0 mL of 0.1 M KCl containing 1.0 mM $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ (1:1 mixture) at a scan rate of 50 mV/s. Differential pulse voltammetry (DPV) was performed by scanning the potential in the range from −1.2 to 0.3 V at a differential pulse step potential of 0.005 V and a modulation amplitude of 0.05 V.

2.5. ELISA and LC–MS measurements

The ELISA measurements of feedstuff specimens were performed similarly to previously used analyses. Briefly, the procedure began with 37 °C coating of 96-well plates by clenbuterol-BSA (100 ng/well) for 60 min followed by 60 min of blocking of excess binding sites with BSA (3%). Produced in PBS containing Tween-20 (0.05%), the competing clenbuterol antigen went through 45 min incubation with the primary antibody at 2.0 μg/mL. With an *o*-phenylenediamine (OPD) substrate, color evolution was achieved succeeding 60 min incubation with HRP-tagged anti-mouse-IgG. To detect clenbuterol levels in feed specimens, this work employed a clenbuterol calibration profile.

A Waters Symmetry^{TM} C_{18}(3.5 μm; 2.1 mm × 150 mm) column provided the platform to chromatographically separate clenbuterol. Aqueous ammonium formate (0.01 M, pH 3.8) serving as mobile phase A and acetonitrile as mobile phase B were transported at a flow rate of 0.2 mL min^{-1}. This work employed a gradient program beginning with 98/2 A/B (v/v), shifting linearly to 70/30 over 5 min, followed by 50/50 from 5 to 15 min. Prior to the following injection, there was a 10 min equilibration period. Settings of 3.0 kV, 5 V and 25 V, respectively, for the capillary, extractor and cone voltages were optimal conditions for ionization to achieve mass spectrometric determination. Temperatures of 120 and 350 °C, respectively, were set for the ion source and desolvation. The electrospray ionization positive (ESI^+) nebulizing gas was highly purified nitrogen, with ESI^+ mode adjusted for the testing of ionization. A chosen ion mode was employed to monitor clenbuterol's chosen daughter mass ions (*m/z* 203 and 259) as well as parent ion (*m/z* 277) in mild conditions for ionization, with quantitative calculation addressed via abundance of the last ion. An external criterion was employed for the quantification of clenbuterol that was spiked in feed specimens.

3. RESULTS AND DISCUSSION

This is the first use of potassium ferrocyanide solution for the evaluation of the electrochemical performance of decorated GR/Au nanocomposite electrodes. The original GCE and GR/Au/GCE were characterized via cyclic voltammogram (CV) profiles in KCl (0.5 M) containing $[Fe(CN)_6]^{3-/4-}$ aqueous solution (0.01 M), as indicated in Fig. 1A. AuNPs is a kind of well known bio-nanomaterials

because of their large specific surface area, strong adsorption ability, well suitability and good conductivity [39-41]. It can strongly interact with biomaterials and has been utilized as an intermediator to immobilize biomolecule to efficiently retain its activity and to enhance current response in the construction of an electrochemical biosensor. Compared with the original GCE, the GR/Au/GCE displayed a smaller redox peak pair, as indicated in the corresponding CVs. A weaker current signal appeared, since negatively charged $[Fe(CN)_6]^{3-/4-}$ ions were repelled by observable negative charges on nano-Au and GR nanocomposites. As indicated in Fig. 1B, a positively charged redox probe, $Ru(bpy)_3^{2+,}$ was employed to investigate the CVs of the as-prepared electrodes, thus studying the charge on such electrodes. Due to appealing electrostatic forces, there was an improvement in the $Ru(bpy)_3^{2+}$-involved electron-shift reaction on the negatively charged electrode surface compared with that observed for the negatively charged redox probe $[Fe(CN)_6]^{3-/4-}$. In comparison with the original GCE, the GR/Au/GCE witnessed a 3-fold increased $Ru(bpy)_3^{2+}$ (1 mM) response in Na_2SO_4 (0.2 M). The existence of the negatively charged ions on the as-modified electrode surface was confirmed by the above results.

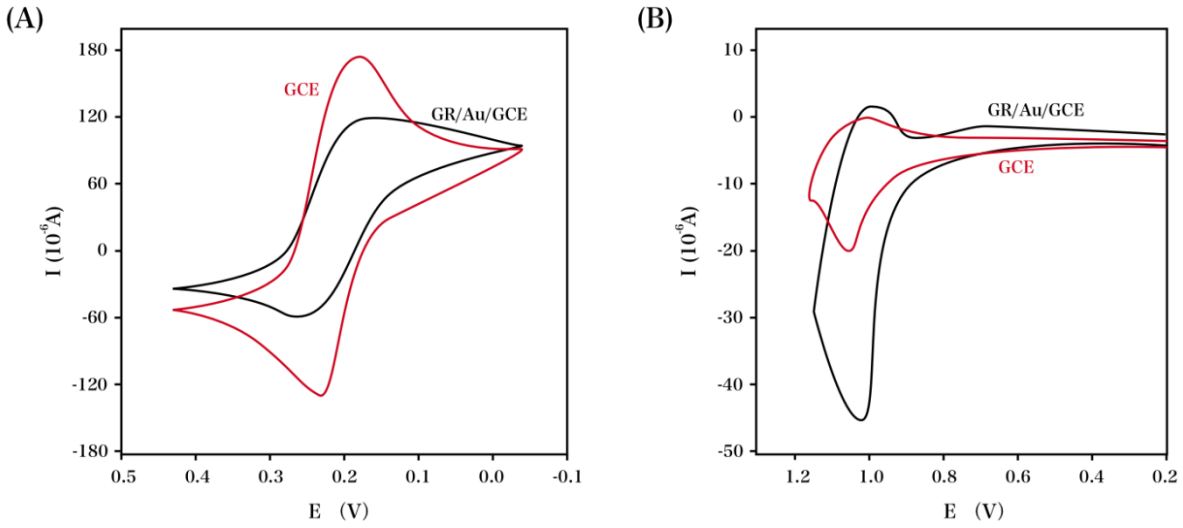

Figure 1. (A) Cyclic voltammograms obtained for a bare GCE and GR/Au/GCE in 0.5 M KCl with 0.01 M $[Fe(CN)_6]^{3-/4-}$. (B) Cyclic voltammograms obtained for a bare GCE and GR/Au/GCE in 0.2 M Na_2SO_4 with 0.01 M mol $Ru(bpy)_3^{2+}$. Scan rate: 50 mV/s.

First, the influence that the temperature exerts on immunoreactions was examined. The studies of antibody-GR/Au reaction were conducted at 50 and 37 °C. The antibody has a strong affinity for CH_{anti} at 37 °C, which is also the temperature of the maximal response. Therefore, 37 °C was selected as the incubation temperature. This resulted has been consisted with previous reports [42, 43]. With respect to specific GR/Au–CH_{anti} recognition, another significant factor is incubation time. The antigens are completely captured on the surface of electrode, as displayed by the electrochemical response increases with lengthened incubation time (20, 40, 60, 80 and 100 min) and the stable stage it achieves after 60 min. This plateau indicates that the antibody-antigen binding sites are saturated by lengthened incubation, leading to an increased non-specific signal. Hence, 60 min was selected as the

optimized incubation time in terms of immunoreactions. Based on previous reports [44, 45], the electrochemical sensor should considered the preparation time due to the practical application requirement. In our case, 60 min incubation period can be adopted for extending the application in many fields such as supermarket, butcher shop etc. The influences that the analytic solution and GR/Au–CH_{anti} concentrations exerted on the electrochemical response were also studied. As indicated in Fig. 2A, an analytical cell (10 mL) containing KNO_3 (0.6 M) and KCL (1.0 M) provided the platform for electrochemical determination. Since there was a Ag/AgCl solid-state voltammetric peak in KCl, significant sensitive clenbuterol determination could be achieved more desirably by the stripping peak (sharp and clear-cut) concerning Ag than that of KNO_3. The fabrication of the on-electrode immunocomplex responding to clenbuterol (modified on GR/Au) quantity is critical to the immunosensor sensitivity. DPV would be boosted in intensity as more immunocomplexes were produced with the increasing number of clenbuterol molecules on GR/Au. Nevertheless, as indicated in Fig. 2B, both the DPV signal and the Au- GR absorption would be diminished by an excess quantity of tagged clenbuterol. The synthesis and use of clenbuterol (at diverse concentrations) modified on GR/Au were used for immunocomplex fabrication to attain optimized immunosensor behavior. A clenbuterol concentration of 100 μg/mL yielded the maximum signal. Hence, for GR/Au–clenbuterol conjugate synthesis to fabricate the immunosensor, this work adopted clenbuterol with a concentration of 100 μg/mL.

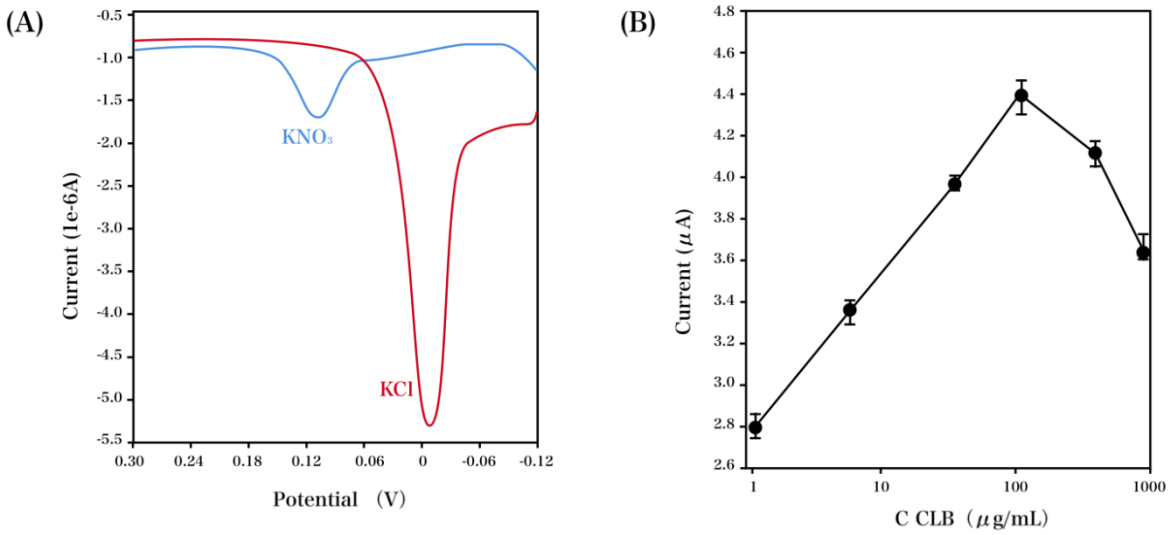

Figure 2. Effect of (A) analytical solutions of KNO_3 and KCl and (B) concentrations of GR/Au–clenbuterol on DPV peak currents.

The immunosensor electrochemical responses to clenbuterol at varied concentrations were investigated under the optimal conditions. As indicated in Fig. 3A, clenbuterol concentration was linearly related to current within the range of 0.01–15.0 ng/mL with a 0.99 correlation coefficient and a DL of 7.2 pg/mL. Herein, 3σ of the blank signal was taken as the basis for DL calculation. To allow realistic comparison with previous reports, the characteristics of different electrochemical sensors for clenbuterol are summarized in Table 1. The interferences of salbutamol, dobutamine and ractopamine

were evaluated to examine the electrochemical immunosensor's specificity for real specimen determination. The as-prepared immunosensor's suitability for real specimen determination and desirable selectivity were demonstrated by the absence of significant interference (Fig. 3B). Inter- and intra-assay coefficients of variation were used to evaluate the immunosensor reproducibility. For 1.0 ng/mL clenbuterol, the intra-assay precision of this technique was 3.6%, while for 0.1 ng/mL clenbuterol, it was 4.2%. Additionally, for 1.0 ng/mL clenbuterol, inter-assay coefficients of variation for five immunosensors were 4.1%, while for 0.1 ng/mL clenbuterol, they were 2.6%, which is superior to those of some of the electrodes reported previously [46-49].

The repeatability and reproducibility of the sensor were evaluated by DPV under the optimum conditions. One GR/Au–CH_{anti} was evaluated by determining 3 ng/mL clenbuterol five times. Given that the relative standard deviation (RSD) was 2.4%, GR/Au–CHanti showed high repeatability. The stability of the modified electrode was evaluated using an amperometric technique for 120 min of continuous operation. At 3 ng/mL clenbuterol, the current at GR/Au–CH_{anti} retained more than 97.9% of its original value. Moreover, five modified electrodes prepared independently managed to determine 3 ng/mL clenbuterol with an RSD of 3.1%.

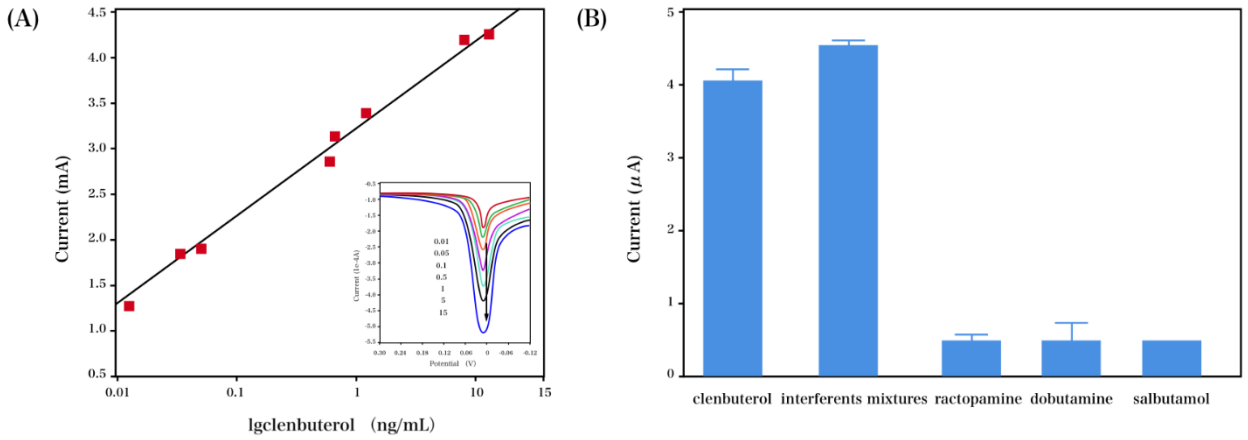

Figure 3. (A) Calibration curves of the immunosensor for clenbuterol determination in 1.0 M KCl. (B) Specificity investigation of the immunosensor for clenbuterol and interferent mixture with clenbuterol (0.1 mg/mL): ractopamine (RAC) (0.1 mg/mL), dobutamine (DOB) (0.1 mg/mL), and salbutamol (SAL) (0.1 mg/mL).

Table 1. Comparison of the major characteristics of electrochemical sensors used in the determination of clenbuterol.

Electrode	Linear detection range	Detection limit	Reference
AgPd NPs/RGO	0.01–100 ng/mL	1.44 pg/mL	[50]
ZnSQD@PANI	0.01–10.0 ng/mL	5.5 pg/mL	[51]
Platinum-nickel-iron/carbon nanosheets	1.9–47 ng/mL	0.7 ng/mL	[52]
GR/Au–CH_{anti}	0.01–15.0 ng/mL	7.2 pg/mL	This work

The evaluation of as-prepared immunosensor's accuracy in real beef specimens was achieved via a recovery study. The beef specimens were acquired from a local market. All the obtained beef specimens were chopped, extracted in ethanol, double-filtered (0.22 μm), and stored in the refrigerator at 4 °C if not used immediately. Clenbuterol standard solution was mixed into blank urine specimens, with 5, 10 and 20 mg/kg obtained as the terminal clenbuterol concentrations in beef specimens. LC-MS, ELISA and amperometric immunosensor were employed with respect to each specimen. The electrochemical immunosensor was desirably consistent with LC-MS and ELISA, as indicated by the results. Clenbuterol at various concentrations was mixed into urine specimens for accuracy evaluation, together with recovery measurement. As indicated by Table 2, the as-prepared sensor was desirably accurate and could be adopted as a parallel method for real specimens, as indicated by the absence of statistical variations with respect to recoveries for the same concentration via the aforementioned three diverse techniques. Our results also comparable with some recent developed clenbuterol sensor [53, 54].

Table 2. Determination of clenbuterol in real beef samples using three methods.

Added (mg/kg)	Immunosensor			ELISA			LC-MS		
	Found (mg/mL)	Recovery (%)	RSD (%)	Found (mg/mL)	Recovery (%)	RSD (%)	Found (mg/mL)	Recovery (%)	RSD (%)
5	4.87	97.4	1.2	4.89	97.8	7.9	5.10	102.0	8.2
10	10.07	100.7	5.5	9.58	95.8	4.4	9.21	92.1	1.6
20	19.02	95.1	3.6	20.21	101.05	3.6	21.55	107.75	5.5

4. CONCLUSION

To conclude, in order to super-sensitively determine clenbuterol, this work proposed a facile and super-sensitive electrochemical immunosensor. Herein, with the role of nanocarrier taken by GR for Au NP and clenbuterol immobilization, the sensitivity was boosted, and clenbuterol tagging was addressed by GR/Au nanocomposites employed as labels. A linear response (0.1–15 ng/mL) with a DL of 7.2 pg/mL was obtained for the as-prepared electrochemical immunosensor on the basis of an excellent immunoreaction technique. Clenbuterol has the potential to be conveniently and rapidly measured in real beef specimens via the as-prepared electrochemical immunosensor, which features desirable selectivity, stability, sensitivity and precision.

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References

1. R. Wellenreiter and L. Tonkinson, *Poult. Sci*, 69 (1990) 142.

2. N. Engeseth, K. LEE, W. Bergen, W. Helferich, B. Knudson and R. Merkel, *Journal of Food Science*, 57 (1992) 1060.
3. L. Watkins, D. Jones, D. Mowrey, D. Anderson and E. Veenhuizen, *Journal of Animal Science*, 68 (1990) 3588.
4. D. Anderson, E. Veenhuizen, J. Wagner, M. Wray and D. Mowrey, *J. Anim. Sci*, 67 (1989) 222.
5. J. Blanca, P. Munoz, M. Morgado, N. Méndez, A. Aranda, T. Reuvers and H. Hooghuis, *Anal. Chim. Acta.*, 529 (2005) 199.
6. G. Mitchell and G. Dunnavan, *Journal of Animal Science*, 76 (1998) 208.
7. J. Martinez-Navarro, *The Lancet*, 336 (1990) 1311.
8. C. Pulce, D. Lamaison, G. Keck, C. Bostvironnois, J. Nicolas and J. Descotes, *Veterinary and Human Toxicology*, 33 (1991) 480.
9. H. Kuiper, M. Noordam, M. van Dooren-Flipsen, R. Schilt and A. Roos, *Journal of Animal Science*, 76 (1998) 195.
10. P. Guy, M. Savoy and R. Stadler, *Journal of Chromatography B: Biomedical Sciences and Applications*, 736 (1999) 209.
11. L. Lin, J. Tomlinson and R. Satzger, *Journal of Chromatography A*, 762 (1997) 275.
12. I. Abukhalaf, D. von Deutsch, B. Parks, L. Wineski, D. Paulsen, H. Aboul-Enein and D. Potter, *Biomedical Chromatography*, 14 (2000) 99.
13. L. He, Y. Su, Z. Zeng, Y. Liu and X. Huang, *Animal Feed Science and Technology*, 132 (2007) 316.
14. M. Johansson and K. Hellenäs, *International journal of food science & Technology*, 39 (2004) 891.
15. A. Posyniak, J. Zmudzki and J. Niedzielska, *Anal. Chim. Acta.*, 483 (2003) 61.
16. Y. Chen, W. Wang, J. Duan, H. Chen and G. Chen, *Electroanalysis*, 17 (2005) 706.
17. S. Moane, M. Smyth and M. O'Keeffe, *The Analyst*, 121 (1996) 779.
18. M. Johansson and K. Hellenäs, *Food and Agricultural Immunology*, 15 (2003) 197.
19. I. Traynor, S. Crooks, J. Bowers and C. Elliott, *Anal. Chim. Acta.*, 483 (2003) 187.
20. F. Liu, Y. Zhang, W. Yin, C. Hou, D. Huo, B. He, L. Qian and H. Fa, *Sensors and Actuators B: Chemical*, (2016)
21. G. Lai, H. Cheng, C. Yin, L. Fu and A. Yu, *Electroanalysis*, 28 (2016) 69.
22. L. Shi, Y. Wang, S. Ding, Z. Chu, Y. Yin, D. Jiang, J. Luo and W. Jin, *Biosensors and Bioelectronics*, (2016)
23. R. Li, T. Yang, Z. Li, Z. Gu, G. Wang and J. Liu, *Anal. Chim. Acta.*, 954 (2017) 43.
24. M. Islam, M. Khan, M. Hossain and M. Hasan, *Progress in Natural Science: Materials International*, 26 (2016) 341.
25. Y. Lv, C. Tao, J. Huang, Y. Li, F. Wang, F. Cai and J. Wang, *Nanomaterials and Nanotechnology*, 6 (2016) 1847980416682443.
26. G. Liu, M. Qi, Y. Zhang, C. Cao and E. Goldys, *Anal. Chim. Acta.*, 909 (2016) 1.
27. M. Yang, T. Jiang, Y. Wang, J. Liu, L. Li, X. Chen and X. Huang, *Sensors and Actuators B: Chemical*, 245 (2017) 230.
28. Y. Cheng, H. Fa, W. Yin, C. Hou, D.. Huo, F. Liu, Y. Zhang and C. Chen, *Journal of Solid State Electrochemistry*, 20 (2016) 327.
29. T. Chen, L. Tang, F. Yang, Q. Zhao, X. Jin, Y. Ning and G. Zhang, *Analytical Letters*, 49 (2016) 2223.
30. A. Zhao, Z. Zhang, P. Zhang, S. Xiao, L. Wang, Y. Dong, H. Yuan, P. Li, Y. Sun and X. Jiang, *Anal. Chim. Acta.*, 938 (2016) 63.
31. Y. Zhu, D. Pan, X. Hu, H. Han, M. Lin and C. Wang, *Sensors and Actuators B: Chemical*, 243 (2017) 1.
32. X. Liu, J. Tong, Z. Yuan, Y. Yang, C. Mao, H. Niu, B. Jin and S. Zhang, *Journal of Nanoscience and Nanotechnology*, 16 (2016) 1645.
33. M. Mashhadizadeh, A. Azhdeh and N. Naseri, *Journal of Electroanalytical Chemistry*, 787 (2017) 132.

34. C. Zou, B. Yang, D. Bin, J. Wang, S. Li, P. Yang, C. Wang, Y. Shiraishi and Y. Du, *Journal of Colloid and Interface Science*, 488 (2017) 135.
35. X. Lu, P. Wang, X. Wang and Y. Guo, *Int. J. Electrochem. Sci*, 11 (2016) 5279.
36. C. Shen, C. Hui, T. Yang, C. Xiao, J. Tian, L. Bao, S. Chen, H. Ding and H. Gao, *Chemistry of Materials*, 20 (2008) 6939.
37. H. Afsharan, B. Khalilzadeh, H. Tajalli, M. Mollabashi, F. Navaeipour and M. Rashidi, *Electrochimica Acta*, 188 (2016) 153.
38. Y. Ye, J. Gao, H. Zhuang, H. Zheng, H. Sun, Y. Ye, X. Xu and X. Cao, *Microchim. Acta.*, 184 (2017) 245.
39. Z. Liu, Y. Yang, H. Wang, Y.-L. Liu, G. Shen and R. Yu, *Sensors and Actuators B: Chemical*, 106 (2005) 394.
40. Y. Xiao, F. Patolsky, E. Katz, J.F. Hainfeld and I. Willner, *Science*, 299 (2003) 1877.
41. K. Huang, D. Niu, X. Liu, Z. Wu, Y. Fan, Y. Chang and Y. Wu, *Electrochimica Acta*, 56 (2011) 2947.
42. P. Miao, K. Han, H. Sun, J. Yin, J. Zhao, B. Wang and Y. Tang, *ACS Applied Materials & Interfaces*, 6 (2014) 8667.
43. H. Zhai, Z. Liu, Z. Chen, Z. Liang, Z. Su and S. Wang, *Sensors and Actuators B: Chemical*, 210 (2015) 483.
44. F. Yan, Y. Zhang, S. Zhang, J. Zhao, S. Liu, L. He, X. Feng, H. Zhang and Z. Zhang, *Microchim. Acta.*, 182 (2015) 855.
45. Y. Dou, Z. Jiang, W. Deng, J. Su, S. Chen, H. Song, A. Aldalbahi, X. Zuo, S. Song and J. Shi, *Journal of Electroanalytical Chemistry*, 781 (2016) 339.
46. P. Andrea and M. Stanislav, *Sensors and Actuators B: Chemical*, 76 (2001) 286.
47. M. Ganjali, P. Norouzi, M. Ghorbani and A. Sepehri, *Talanta*, 66 (2005) 1225.
48. M. Rajkumar, Y. Li and S. Chen, *Colloids and Surfaces B: Biointerfaces*, 110 (2013) 242.
49. Z. Cao, Y. Zhao, Y. Dai, S. Long, X. Guo and R. Yang, *Sensor Letters*, 9 (2011) 1985.
50. H. Wang, Y. Zhang, H. Li, B. Du, H. Ma, D. Wu and Q. Wei, *Biosensors and Bioelectronics*, 49 (2013) 14.
51. Z. Zhang, F. Duan, L. He, D. Peng, F. Yan, M. Wang, W. Zong and C. Jia, *Microchim. Acta.*, 183 (2016) 1089.
52. Y. Feng, S. Niu, X. Fu, J. Zhao and Y. Yang, *Chinese Journal of Analytical Chemistry*, 3 (2013) 022.
53. Y. Yang, H. Zhang, C. Huang, D. Yang and N. Jia, *Biosensors and Bioelectronics*, 89, Part 1 (2017) 461.
54. L. Wang, R. Yang, J. Chen, J. Li, L. Qu and P. Harrington, *Food Chemistry*, 164 (2014) 113.