Int. J. Electrochem. Sci., 7 **(2012)** 9150 - 9160

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electroanalysis, Electrodeposition and Electrochemical Characterization of Ractopamine

Ying Li, Shen-Ming Chen[*]

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No.1, Section 3, Chung-Hsiao East Road , Taipei 106, Taiwan (R.O.C).
[*]E-mail: smchen78@ms15.hinet.net

Received: 20 August 2012 / *Accepted:* 11 September 2012 / *Published:* 1 October 2012

We have first reported the modification of ractopamine electrodeposition films. In this paper, the electrochemical oxidation of ractopamine in different pHs and conditions at the bare glassy carbon electrode. The bare glassy carbon electrode also exhibits a promising enhanced electroanalytic activity towards the oxidation of ractopamine. Different methods were used for the formation of poly-ractopamine films and the deposition on electrode. Cyclic voltammograms (CVs), Linear sweep voltammetry (LSV) and Electrochemical impedance spectroscopy (EIS) are used for the determination of ractopamine and the apparent diffusion coefficient values for these compounds at different concentration as it gives some information about the kinetics of charge transfer during the redox reactions of these compounds. Finally, we have studied the surface morphology of the modified electrode using atomic force microscopy (AFM), which revealed that ractopamine is coated on ITO.

Keywords: Ractopamine, Modified electrodes, Drug Analysis, Electroanalysis, Electrochemical, Electrodeposition.

1. INTRODUCTION

Ractopamine (RAC) is a β-adrenergic agonist (β-agonist) which at repartitioning doses has been found to increase muscle growth and decrease fat deposition in different animal species [1-6]. RAC is widely used in swine, cattle, and turkeys as a feed additive to increase feed efficiency and carcass leanness by inhibiting fat production, stimulating lipolysis, and increasing protein synthesis [7-8]. Paylean® is the brand name of the swine feed. It is an orally active compound that can be directly added to swine diets. The primary function of RAC is to increase the energy consumed by the animal so as to increase carcass muscle growth and decrease fat tissue growth. Because lean tissue growth

requires less energy than fat tissue growth, pigs fed Paylean achieve increased growth rates and better feed efficiency relative to conventionally raised ones. Research by animal scientists has found that when swine are fed a constant dietary Paylean concentration, carcass lean and bodyweight growth responses peak shortly after commencing supplementation and then slowly decline. The results of this study suggest that the most significant changes associated with the use of repartitioning doses of RAC in pigs involve the endocrine glands and the male lower urinary tract. The lesions observed in prostate and urethral epithelium can be considered as adaptative reversible lesions induced by RAC treatment. The role of RAC in the determinism of eosinophilic infiltration in lymphoid organs and in the ovary of gilts remains to be clarified [9].

Under most combinations of health status and pricing scheme, adjusting the Paylean concentration in the hog feed proved to be profitable relative to a constant concentration. Thus, it is shown that ignoring the desensitizing potential of chemical inputs in biological production could result in economic loss [10]. RAC is an Association of Racing Commissioners International class 3 drug and is not recognized as a therapeutic medication in racing animals [11]. It has considerable potential for illegal use in humans and show and performance animals because it may have the ability to significantly affect performance via its β-adrenergic agonist properties and its anabolic activities [12-13]. This is the first reported case of arterial, cardiac, and skeletal muscle damage associated with ractopamine [14]. This risk is based on potential for adverse cardiovascular and nervous system effects associated with β-agonist overdose.

Although RAC was approved as a pig feed supplement in 1999 by the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine, and subsequently by regulatory officials in Brazil, Venezuela, Colombia, Guatemala, Philippines, etc., many countries in Asia and Europe have not licensed to use it as a repartitioning agent, for its possible adverse effects on human health by carry-over from RAC-treated animals to human diet [15-16]. Therefore, a rapid, selective and reproducible survey method would be useful in order to determine of RAC. Different methods such as enzyme-linked immunosorbent assay (ELISA) [17-20], high-performance liquid chromatography (HPLC) [21-23], surface plasmon resonance (SPR) [24], gas chromatography–mass chromatography (GC-MS) [25] and liquid chromatography–tandem mass chromatography (LC-MS/MS) [26-29], have been developed for screening and confirmatory determination of RAC in feeds, animal tissues and urine, milk, etc. However, in the clean-up step, most of them used the conventional solid-phase extraction technique, which shows a lack of selectivity and specificity, so that a large amount of matrix interferences are eluted simultaneously with the target analyte. Electrochemical methods [30-41], such as amprometric biosensors have been extensively employed for determination of RAC for their simplicity, highly selective and intrinsic sensitivity. This paper discusses the electrochemical polymerization of RAC films. It was interesting to study the electrochemical oxidation of RAC in different pHs and conditions. In addition, the observed behavior and reactions from RAC have been estimated by digital simulation of cyclic voltammograms (CVs) and electrochemical impedance spectroscopy (EIS).

2. EXPERIMENTAL

2.1. Materials

Ractopamine (Aldrich) was used as received. All other chemicals used were of analytical grade and used without further purification pH 7.0 (0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4) Phosphate buffer solutions (PBS), pH 1.0 H_2SO_4 solutions and pH 11 KOH buffer were used as supporting electrolyte. Aqueous solutions were prepared using doubly distilled deionized water and then deaerated by purging with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements.

2.2. Apparatus

Cyclic voltammetry (CVs) and Linear sweep voltammetry (LSV) were performed in an analytical system model CHI-1205A potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy carbon electrode (GCE; area 0.07 cm^2). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. The morphological characterizations of the films were examined by atomic force microscopy (AFM) (Being Nano-Instruments CSPM5000). Electrochemical impedance spectroscopy (EIS) measurements were performed using an IM6ex Zahner instrument (Kroanch, Germany). All the solutions were purged with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements. All the experiments were carried out at room temperature (≈ 25°C).

2.3. Preparation of ractopamine modified electrodes by electrodeposition

Prior to modification, glassy carbon electrode (GCE) was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature. The electropolymerization of ractopamine was done by electrochemical oxidation of ractopamine (1 mg/ml) on the glassy carbon electrode using pH 1.0 H_2SO_4 buffer. It was performed by consecutive cyclic voltammetry over a suitable potential range of -0.2 to 1.3 V; scan rate 100 mVs^{-1} for 20 cycles. The optimization of poly-ractopamine growth potential has been determined by various studies with different electrodeposition(or electropolymerization) potentials.

3. RESULTS AND DISCUSSIONS

3.1. Electrochemical characterizations of ractopamine in different pH

The electrochemical properties of ractopamine at a glassy carbon electrode were investigated

using cyclic voltammetry in aqueous solutions having pH values between 1 and 11. Figure 1 (A) to (F) showed the cyclic voltammetric of baer glassy carbon electrode obtained in various pH aqueous solution containing ractopamine (1 mg/ml), scan rate = 100 mVs^{-1}. Figure (A) in low pH (pH 1.0) response of oxidation process resulted in irreversible oxidation peak about 985 mV. (B) to (F) in pH 3.0 to 11 showed that the peak potentials shifted to the negative potentials by increasing pH. Exhibited of oxidation process produced irreversible peak. Figure (F) showed pH 11 results, the initial stage was similar irreversible oxidation process about 433 mV.

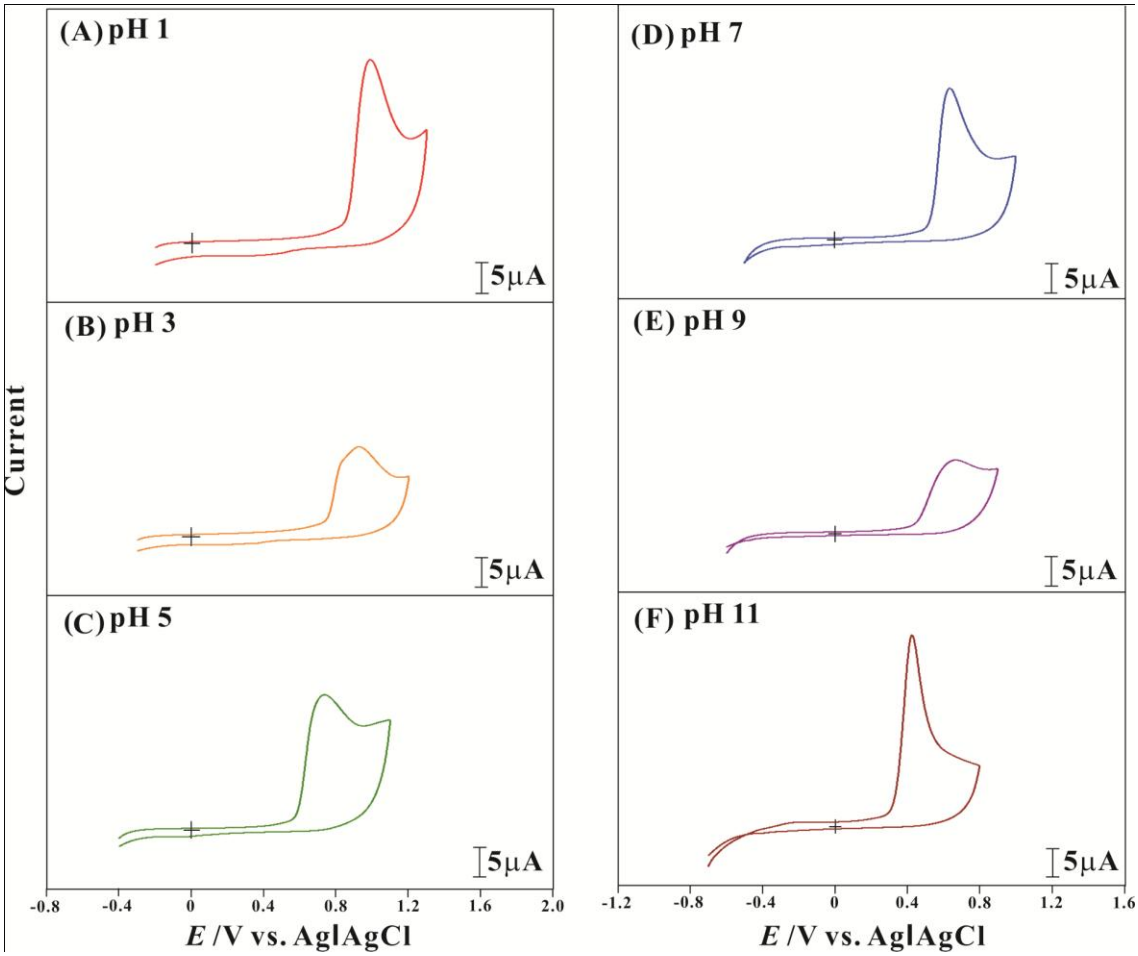

Figure 1. Cyclic voltammograms of the bare glassy carbon electrode transferred to various pH solutions containing ractopamine (1 mg/ml) (A) 1; (B) 3; (C) 5; (D) 7; (E) 9; (F) 11. Scan rate100 mVs^{-1}.

Figure 2 revealed in pH 1.0 oxidation of ractopamine. In first segment, this is expected because of the participation of proton(s) in the oxidation reaction of ractopamine for two-electron process. There is a direct relation between ractopamine oxidation and glucuronide conjugation in animals. Glucuronic acid is attached via a glycosidic bond to the ractopamine, and the resulting glucuronide, which has a much higher water solubility than the original ractopamine, is eventually excreted by the kidneys. Glucuronide conjugation is a process that animals use to assist in the drugs, excretion of

toxic substances and other lipid substances that cannot be used as an energy source [20, 24,42-43].

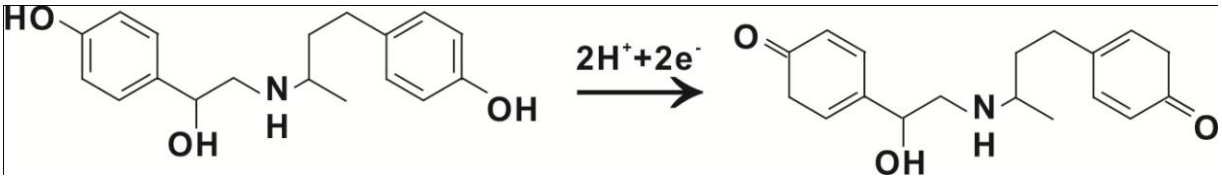

Figure 2. Ractopamine oxidation process.

3.2. Electroanalysis characterizations of ractopamine

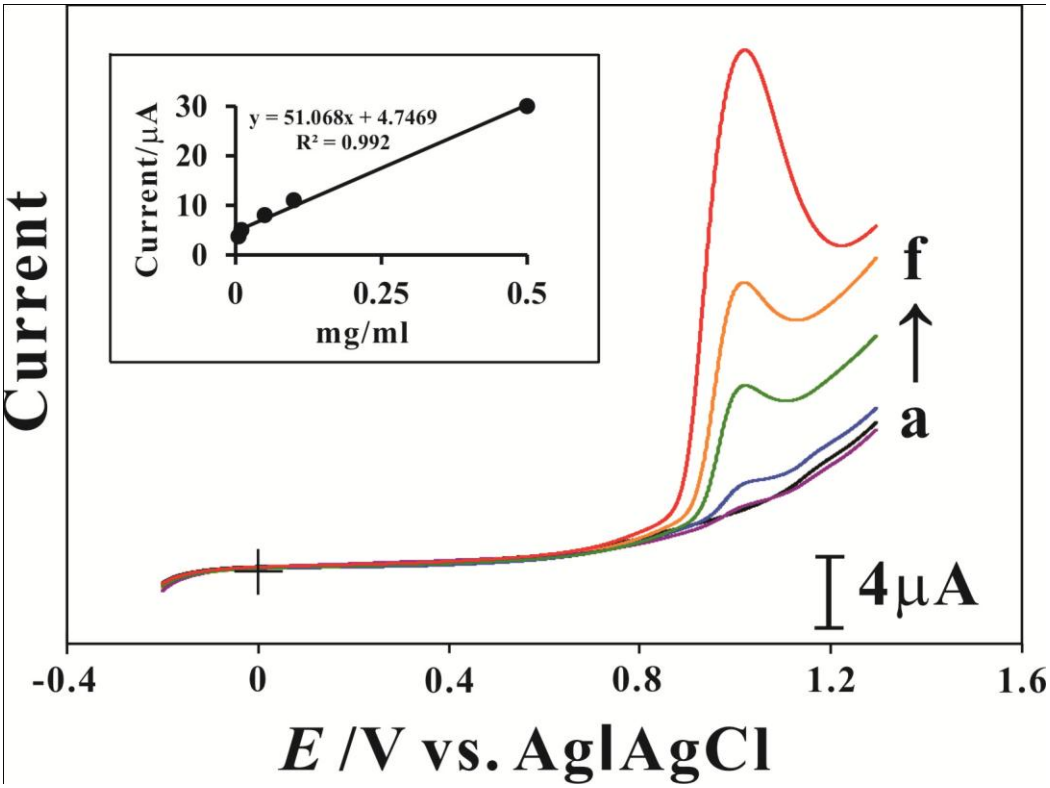

Figure 3. Linear sweep voltammetry (LSV) of bare glassy carbon electrode in pH 1 H_2SO_4 buffer with various concentrations of ractopamine : (a) 0; (b) 0.005; (c) 0.01; (d) 0.05; (e) 0.1 and (f) 0.5 mg/ml. The inset shows the plot of current versus concentration of ractopamine.

The electroanalytic oxidation efficiency of baer glassy carbon electrode in the absence and presence of different concentration ractopamine was investigated using Linear sweep voltammetry (LSV). Figure 3 showed the baer glassy carbon electrode deposition in pH 1.0 H_2SO_4 aqueous solutions (curve a). Different concentration of ractopamine electroanalytic oxidation showed in curve (b) 0.005; (c) 0.01; (d) 0.05; (e) 0.1 and (f) 0.5 mg/ml. Curve (b) to (f) showed that the gorwing current peak by increased concentration of ractopamine. The response of sensitivity and correlation coefficient were 729.54 μA $(mg/ml)^{-1}$ cm^2 and R^2= 0.992.

3.3. Electrodeposition of ractopamine modified electrode

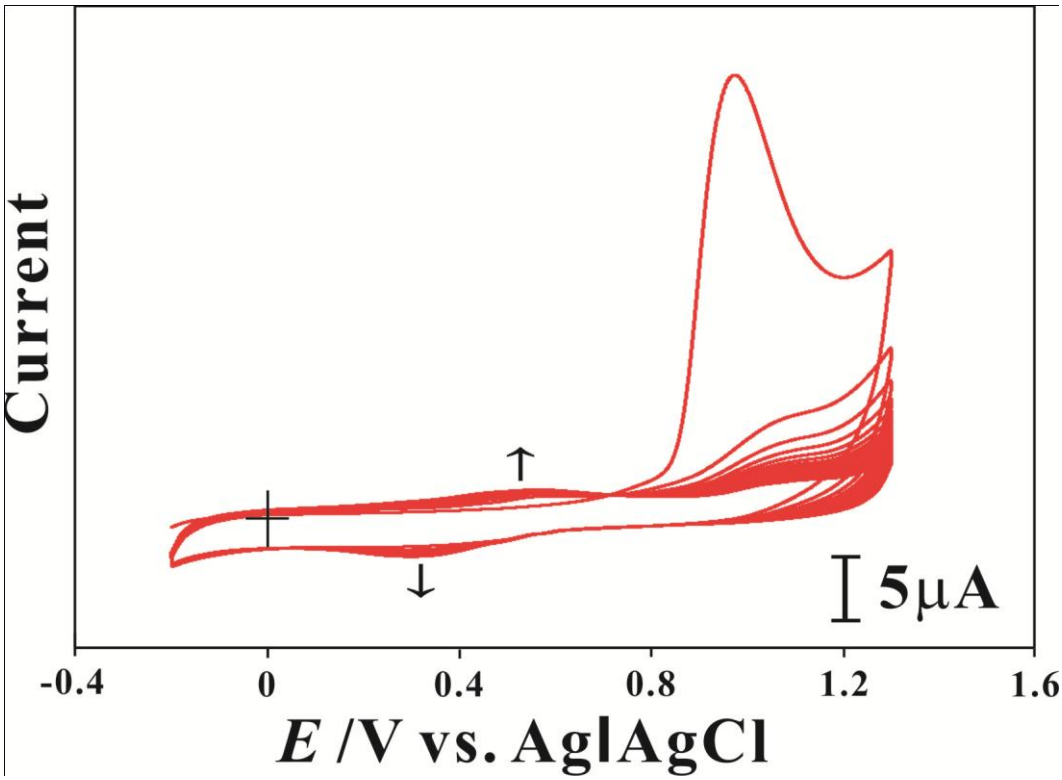

Figure 4. Cyclic voltammograms of bare glassy carbon electrode in pH 1.0 H_2SO_4 buffer containing ractopamine (1 mg/ml) at the potential range of -0.2 to 1.3 V, scan rate at 100 mVs^{-1} for 20 cycles.

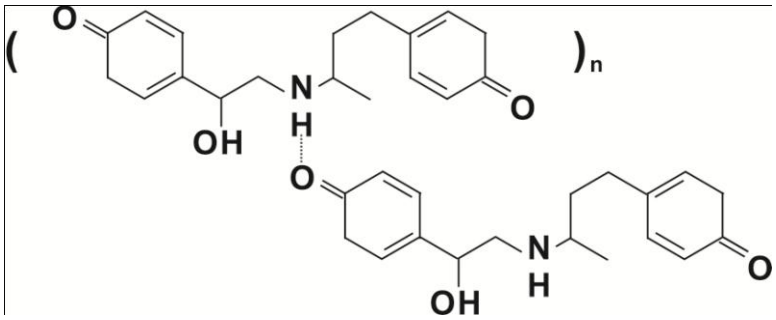

Figure 5. Electrodeposition of ractopamine.

Figure 4 showed the electropolymerization of ractopamine (1 mg/ml) by electrochemical oxidation on glassy carbon electrode using pH 1.0 H_2SO_4 buffer. It was performed by consecutive cyclic voltammogram over a suitable potential range of -0.2 to 1.3 V; scan rate = 100 mVs^{-1}. The growth of the cyclic voltammogram current exhibiting a redox couple with a formal potential of $E^{0'}$ = 302 mV and 553 mV (vs. Ag|AgCl). The increase in peak current at the redox couple indicates that film formation occurred. Ractopamine modified films could also be synthesized in strong acidic aqueous solutions using consecutive cyclic voltammetry on indium tin oxide (ITO) electrodes that had

been modified. Electrodeposition of ractopamine process as in Figure 5. In the following experiments, each newly prepared film on glassy carbon electrode has been washed carefully in deionized water to remove the loosely bounded ractopamine on the modified glassy carbon electrode. It was then transferred to different aqueous solution for the other electrochemical characterizations.

3.4. Electrochemical impedance spectra (EIS) of ractopamine

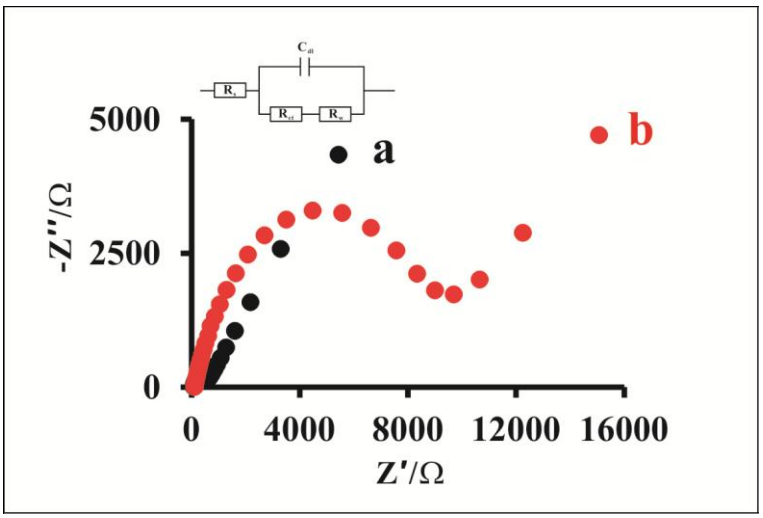

Figure 6. Electrochemical impedance spectra (EIS) of (a) bare electrode and (b) ractopamine modified electrode in pH 7.0 PBS containing 5×10^{-3} M $[Fe(CN)_6]^{-3/-4}$, amplitude: 5 mV. The inset displayed the equivalent circuit (Randles model) was used to fit Nyquist diagrams.

Electrochemical Impedance Spectroscopy (EIS) is a powerful diagnostic tool that you can use to characterize limitations and improve the performance of biosensor. There are some fundamental value sources of electron transfer resistance (R_{et}), double layer capacity (C_{dl}), electron-transfer kinetics and diffusion. The plot of the real component (Z′) and the imaginary component Z″ (imaginary) resulted in the formation of a semicircular Nyquist plot. This type of impedance spectrum is an analytic of a surface-modified electrode system in which the electron transfer is slow and the impedance is controlled by the interfacial electron transfer at high frequency. By applying physically-sound equivalent circuit models wherein physiochemical processes occurring within the biosensor are represented by a network of resistors, capacitors and inductors, you can extract meaningful qualitative and quantitative information regarding the sources of impedance within the biosensor. EIS is useful for research and development of new materials and electrode structures, as well as for product verification and quality assurance in manufacturing operations. Figure 6 showed the results of EIS for different modified electrodes in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$. The Faradaic impedance spectra, presented as Nyquist plots (Z″vs. Z′) for the bare and poly-ractopamine modified electrodes. The bare electrode exhibited almost a straight line (curve a) with a very small depressed semicircle arc (R_{et} = 266 (Z′/Ω)) represents the characteristics of diffusion limited electron-transfer process on the electrode surface. On the same conditions, the poly-ractopamine modified electrode

(curve b) shows like a depressed semicircle arc (R_{et} = 9708 (Z'/Ω)) clearly indicated the higher electron transfer resistance behavior comparing with bare electrode. The most common and simplest model fitted to EIS spectra of electrochemical behavior is a simplified Randle's model shows in inset. It constitutes a distributed element which can only be approximated by an infinite series of simple electrical elements.

3.5. Different scan rate studies

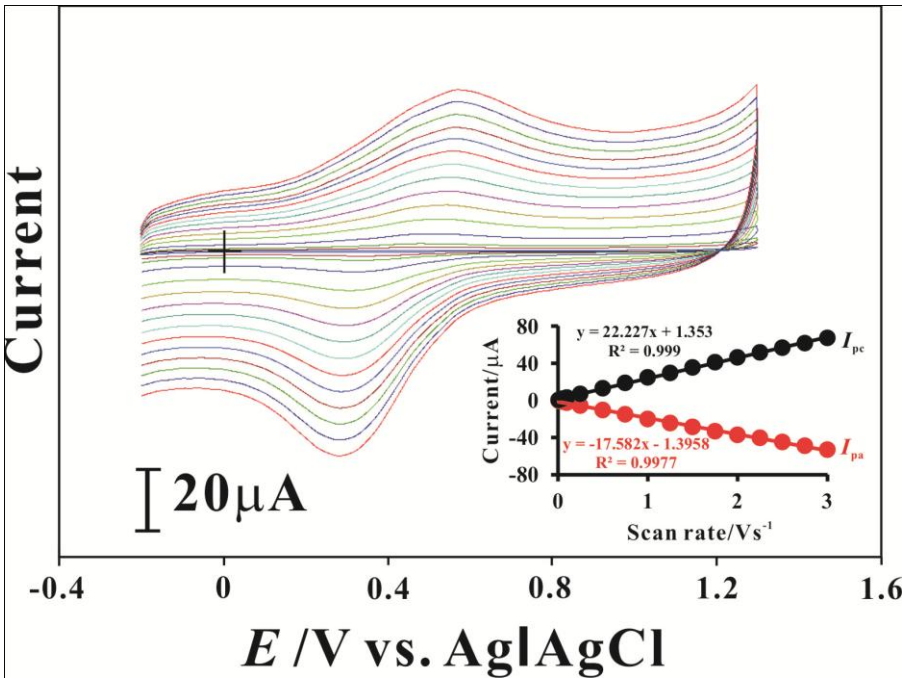

Figure 7. Cyclic voltammogarms of ractopamine modified electrode in pH 1.0 H_2SO_4 buffer different scan rate from 10 mVs^{-1} to 3000 mVs^{-1}, respectively. Calibration curve for data showed I_{pa} & I_{pc} vs. scan rate.

Figure 7 showed that the poly ractopamine on a glassy carbon electrode had one chemically reversible redox couple at 301 and 584 mV in the pH 1.0 aqueous H_2SO_4 solution when cyclic voltammetry was performed at different scan rates (10 to 3000 mVs^{-1}). The anodic and cathodic peak currents of both the film redox couples which have increased linearly with the increase of scan rates. The calibration curve for data showed I_{pa} & I_{pc} vs. scan rate. The ratio of I_{pa}/I_{pc} has demonstrated that the redox process has not been controlled by diffusion. This behavior perhaps occurs because of a reversible electron transfer process involving the poly-ractopamine layer, with a proton exchange process occurring along with the electron transfer process. However, the ΔE_p of each scan rate reveals that the peak separation of composite redox couple increases as the scan rate is increased.

3.6. Morphological characterization of ractopamine

The surface morphology of poly ractopamine modified electrode has been examined using

AFM. Here the AFM studies could furnish the comprehensive information about the surface morphology of nanostructure on the ITO surface. In prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15 min and then dried. The AFM parameters have been evaluated from 1000×1000 nm and 20000×20000 nm surface area. Further, three different thickness ractopamine modified films (A) 5, (B) 10 and (C) 20 cycles by cyclic voltammogarms on ITO modified electrodes were characterized using AFM.

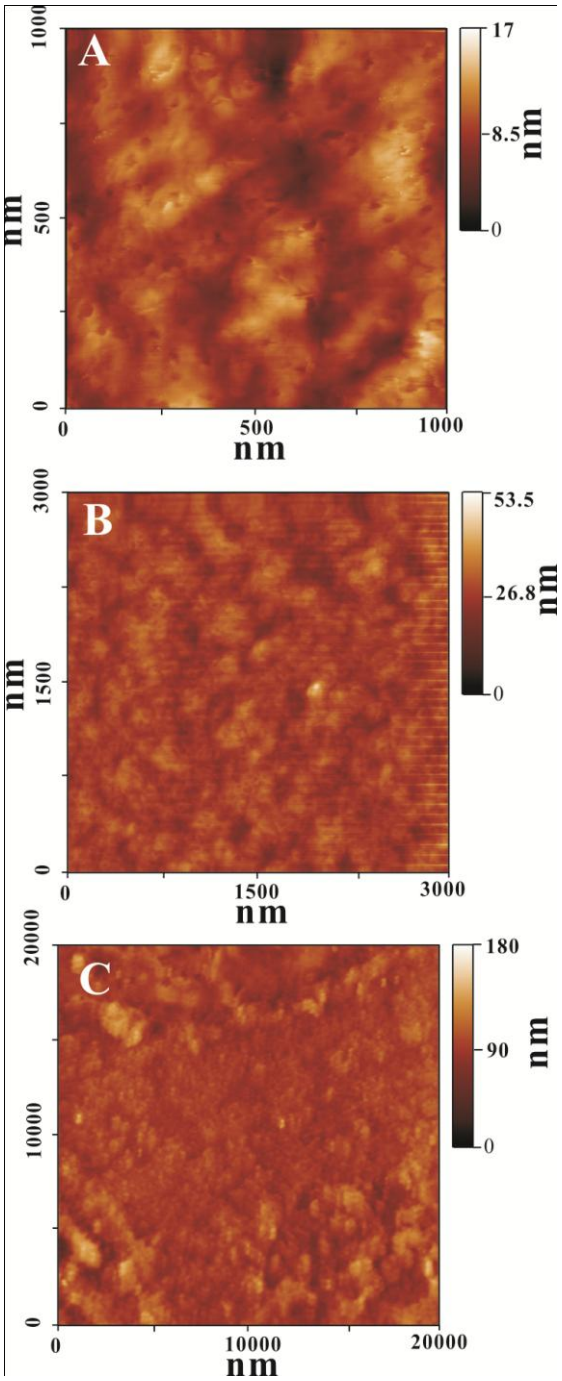

Figure 8. AFM images of different thickness ractopamine modified films (A) 5, (B) 10 and (C) 20 cycles by cyclic voltammogarms on ITO electrode.

From Figure 8, it is significant that there are morphological differences between both the films. The top views of nanostructures (A) shows uniformly deposited homogeneously dispersed ractopamine on this electrode. We can see the existence of nanostructures in obvious manner with the average size range of 7.3 nm. The other amplitude parameters such like roughness average (sa) for acetaminophen film (1000×1000 nm) was found as 1.25 nm. The root mean square roughness was found as 1.55 nm. The poly ractopamine of 10 cycles in Figure 8 (B) reveals that the poly ractopamine had covered more, average size range of 16.8 nm, roughness average (sa) of 2.39 nm and the root mean square roughness of 3.7 nm. Comparison of (A), (B) and (C) reveals, these results in could be explained as the increase in deposition of ractopamine presence 20 cycles. We can clearly see that the immersed poly ractopamine on surface.

4. CONCLUSIONS

We have demonstrated application of the baer glassy carbon electrode for determination of ractopamine. This feature provides a favorable clinical diagnosis for the electroanalytic oxidation of ractopamine at baer glassy carbon electrode. High sensitivity and stability together with very easy preparation bar glassy carbon electrode as promising candidate for constructing simple electrochemical sensor for ractopamine determination. The experimental methods of Cyclic voltammetry (CVs) and Linear sweep voltammetry (LSV) with biosensor integrated into the bare glassy carbon electrode which are presented in this paper, provide an opportunity for qualitative and quantitative characterization, even at physiologically relevant conditions. Preparation of ractopamine modified electrodes showed stable response. The AFM results have shown the difference thickness between 5 to 20 cycles practopamine modified films morphological data. Therefore, this work establishes and illustrates, in principle and potential, a simple and novel approach for the development of a biosensor which is based on the glassy carbon electrode and ITO electrodes.

ACKNOWLEDGEMENT
This work was supported by the National Science Council of the Taiwan (ROC).

References

1. P.K. Baker, R.H. Dalrymple, D.L. Ingle, C.A. Ricks, *J. Anim. Sci.* 59 (1984) 1256-1261.
2. G. Vogel, A.Schroeder, Platter, M. van Koevering, *J. Anim. Sci.* 83 (2005) 112.
3. S.E. Mills, M.E. Spurlock, *J. Anim. Sci.* 81 (2003) 662-668.
4. C.A. Ricks, R.H. Dalrymple, P.K. Baker, D.L. Ingle, *J. Anim. Sci.* 59 (1984) 1247-1255.
5. M.N. Sillence, *Vet. J.* 163 (2004) 242-257.
6. L.E. Watkins, D.J. Jones, D.H. Mowrey, D.B. Anderson, E.L. Veenhuizen, *J. Anim. Sci.* 68 (1990) 3588–3595.
7. P.E. Strydom, L. Frylinck, J.L. Montgomery, *Meat Sci.* 81(2009) 557–564.
8. J.K. Apple, P.J. Rincker, F.K. McKeith, S.N. Carr, T.A. Armstrong, P.D. Matzat, *Pro. Anim. Sci.* 23 (2007) 179–196.

9. D. Catalano, R. Odore, S. Amedeo, C. Bellino, E. Biasibetti, B. Miniscalco, G. Perona, P. Pollicino, P. Savarino, L. Tomassone, R. Zanatta, M.T. Capucchio, *Livestock Science* 144 (2012) 74–81.
10. D. Canchi, N. Li, K. Forter, P. V. Preckel, A. Schinckel, B. Richert, *Amer. J. Agr. Econ.* 92 (2010) 56–69.
11. A.F. Lehner, C.G. Hughes, J.D. Harkins, *J. Anal. Toxicol.* 28 (2004) 226–238.
12. J.G. Burniston, Y. Ng, W.A. Clark, *J. Appl. Physiol.* 93 (2002) 1824–1832.
13. J.G. Burniston, L.B. Tan, D.F. Goldspink., *J. Appl. Physiol.* 98 (2005) 1379–1386.
14. M.J. Yaeger, K. Mullin, S.M. Ensley, W.A. Ware, R.E. Slavin, *Veterinary Pathology* 49 (2012) 569-573
15. H. Yu, S. Yang, *Chin. J. Vet. Drug*. 38 (2004) 44–47.
16. L. He, Y. Su, Z. Zeng, Y. Liu, X. Huang, *Anim. Feed Sci. Tech.* 132 (2007) 316–323.
17. W. Hassnoot, P. Stouten, A. Lommen, *Analyst* 119 (1994) 2675–2680.
18. Shen, L., He, P., *Electrochem. Commun.* 2007, *9*, 657–662.
19. C. Elliott, C. Thompson, C. Arts, S. Crooks, M. Van Baak, E. Verheij, G. Baxter, *Analyst* 123 (1998) 1103–1107.
20. W.L. Shelver, D.J. Smith, *J. Agric. Food Chem.* 50 (2002) 2742-2747.
21. M. Turberg, T. Macy, J. Lewis, *J. Assoc. Off. Annal. Chem. Int.* 77 (1994) 840–847.
22. E. Shishani, S. Chai, S. Jamokha, G. Michael, K. Hoffman, *Anal. Chim. Acta* 483 (2003) 137–145.
23. Q.J. Zhang, Y.J. Su, Q.Q. He, X.G. Shen, L.M. He, N. Zhang, Z.L. Zeng, *J. Sep. Sci.* 34 (2011) 3399–3409.
24. M. Liu, B. Ning, L.J. Qu, Y. Peng, J.W. Dong, N. Gao, L. Liuc, Z.X. Gao, *Sensors Actuat. B* 161 (2012) 124– 130.
25. A. Lehner, C. Hughes, J. Harkins, C. Nickerson, B. Mollett, L. Dirikolu, J. Bosken, F. Camargo, J. Boyles, A. Troppmann, W. Karpiesiuk, W. Woods,T. Tobin, *J. Anal. Toxicol.* 28 (2004) 226–238.
26. J. Antignac, P. Marchand, B. Bizec, F. Andre, *J. Chromatogr. B* 774 (2002) 59–66.
27. J. Blanca, P. Muñoz, M. Morgado, N. Méndez, A. Aranda, T. Reuvers, H. Hooghuis, *Anal. Chim. Acta* 529 (2005) 199–205.
28. B. Shao, X. Jia, J. Zhang, J. Meng, Y. Wu, H. Duan, X. Tu, *Food Chem.* 114 (2009) 1115–1121.
29. C. Li, Y. Wu, T. Yang, Y. Zhang, W.F. Huang, *J. Chromatogr. A* 1217 (2010) 7873–7877.
30. K.C. Lin, X.C. Jian, S.M. Chen, *Int. J. Electrochem. Sci.* 6 (2011) 3427 – 3437.
31. Y. Li, S.M. Chen, W.C. Chen, Y.S. Li, M.A. Ali, F.M.A. AlHemaid, *Int. J. Electrochem. Sci.* 6 (2011) 6398 – 6409.
32. Y. Li, J.X. Wei, S.M. Chen, *Int. J. Electrochem. Sci.* 6 (2011) 3385 – 3398.
33. J.Y. Yang, Y. Li, S.M. Chen, K.C. Lin, *Int. J. Electrochem. Sci.* 6 (2011) 2223 – 2234.
34. Y. Li, C.Y. Yang, S.M. Chen, *Int. J. Electrochem. Sci.* 6 (2011) 4829 – 4842.
35. Y. Li, S.Y. Yang, S.M. Chen, *Int. J. Electrochem. Sci.* 6 (2011) 3982 – 3996.
36. K.C. Lin, T.H. Tsai, S.M. Chen, *Biosens. Bioelectron.* 26 (2010) 608–614.
37. Y. Umasankar, Y. Li, S.Mi. Chen, *J. Electrochem. Soc.* 157 (2010) K187-K193.
38. Y. Li, Y. Umasankar, S.M. Chen, *Talanta* 79 (2009) 486–492.
39. Y. Li, Y. Umasankar, S.M. Chen, *Anal. Biochem.* 388 (2009) 288–295.
40. S. Thiagarajan, T.H. Tsai, S.M. Chen, *Biosens. Bioelectron.* 24 (2009) 2712–2715.
41. A.P. Periasamy, Y.H. Ho, S.M. Chen, *Biosens. Bioelectron.* 29 (2011) 151-158.
42. E.A. Ricke, D.J. Smith,V.J. Feil, G.L. Larsen, J.S. Caton, *J. Anim. Sci.* 77 (1999) 701-707.
43. N.W. Shappell,V.J. Feil, D.J. Smith, G.L. Larsen, D.C. McFarland, *J. Anim. Sci.* 78 (2000) 699-708.