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PII: S0956-5663(15)30612-6
DOI: <http://dx.doi.org/10.1016/j.bios.2015.11.062>
Reference: BIOS8192

To appear in: *Biosensors and Bioelectronics*

Received date: 1 October 2015
Revised date: 19 November 2015
Accepted date: 20 November 2015

Cite this article as: Ming Yan Wang, Wei Zhu, Lin Ma, Juan Juan Ma, Dong En Zhang, Zhi Wei Tong and Jun Chen, Enhanced Simultaneous Detection of Ractopamine and Salbutamol – *via* Electrochemical-Facial Deposition of MnO₂ nanoflowers onto 3D RGO/Ni Foam templates, *Biosensors and Bioelectronics* <http://dx.doi.org/10.1016/j.bios.2015.11.062>

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**Enhanced Simultaneous Detection of Ractopamine and Salbutamol – via
Electrochemical-Facial Deposition of MnO₂ nanoflowers onto 3D RGO/Ni Foam templates**

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Abstract In this paper, we report a facile method to successfully fabricate MnO₂ nanoflowers loaded onto 3D RGO@nickel foam, showing enhanced biosensing activity due to the improved structural integration of different electrode materials components. When the as-prepared 3D hybrid electrodes were investigated as a binder-free biosensor, two well-defined and separate differential pulse voltammetric peaks for ractopamine (RAC) and salbutamol (SAL) were observed, indicating the simultaneous selective detection of both β -agonists possible. The MnO₂/RGO@NF sensor also demonstrated a linear relationship over a wide concentration range of 17 nM to 962 nM ($R=0.9997$) for RAC and 42 nM to 1463 nM ($R=0.9996$) for SAL, with the detection limits of 11.6 nM for RAC and 23.0 nM for SAL. In addition, the developed MnO₂/RGO@NF sensor was further investigated to detect RAC and SAL in pork samples, showing satisfied comparable results in comparison with analytic results from HPLC.

Keywords: β -agonists; MnO₂ nanoflowers; 3D reduced graphene oxide foam; Ni foam; biosensor.

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1. Introduction

β -agonists are synthetic phenethanolamine compounds originally developed for the treatment of pulmonary disease, and also well-known for their ability to improve growth rate and reduce carcass fat when used to feed farm animals in high doses (Suherman et al., 2015). However, the residues of these misused drugs accumulated in animal tissues could cause acute poisoning when consumed by humans, with symptoms of cardiac palpitation, muscle tremors, tachycardia, nervousness, and confusion (Yuping et al., 2015; Thi A.H.N. et al., 2014). Therefore, the use of β -agonists in animal breeding is banned in many countries. Nevertheless, the use of β -agonists remains attractive to swine or cattle producers, because it can improve feed efficiency. Especially now, because of their synergetic effects, some β -agonists are used in combination with other β -agonists as illegal growth promoters in swine and cattle breeding at lower doses. The most common abused β -agonists are ractopamine (RAC) and salbutamol (SAL). Thus, the development of sensitive and selective analytical methods to simultaneously detect RAC and SAL in food is mandatory today.

Up to now, the detection of β -agonists are generally carried out by traditional chromatographic methods, such as HPLC, GC-MS, and HPLC-MS (Guanglong et al., 2015; Limin et al., 2007; Fan et al., 2012; Cun et al., 2010). These chromatographic methods are time consuming and often require sophisticated and large apparatus, making them unsuitable for field routine operation. In recent years, electrochemical assay method has shown great promise in the detection of β -agonists, because most of

the β -agonists can be electrochemically oxidized at bare or modified electrodes (Xiaoyun et al., 2013; Huan et al., 2013) with the advantages of low instrumental cost, fast analysis and low sample consumption.

Nanostructure MnO_2 has drawn great attention as an active electrode material for electrochemical biosensors due to its low cost, environmental benignity, and excellent catalytic and selective ability. A cheap and simple nonenzymatic device for xenoestrogens detection has been developed based on direct precipitation of manganese oxide onto screen-printed carbon electrode (Ana Maria et al., 2015). A MnO_2 ultrathin nanosheets exhibited high electrochemical activity for detection of H_2O_2 (Ping et al., 2014). Through in situ synthesis process, *C-dots-MnO₂* nanocomposites were fabricated for rapid and selective sensing of glutathione (Qiyong et al, 2015). A porous MnO_2/CNT composite electrode was successfully produced by a simple “dipping and drying” process for non-enzymatic glucose detection (Chunyan et al., 2015). One electrodeposition protocol for growing structurally integrated PtAu alloy and MnO_2 on freestanding graphene paper was developed as high-performance flexible electrochemical glucose sensors (Fei et al, 2013). A novel $\text{MnO}_2/\text{polyaniline}$ composites electrode was fabricated for simultaneous detection of guanine, adenine, thymine and cytosine (Prathap et al, 2013). Though nanostructured MnO_2 has shown good electrocatalytic properties as mentioned above, it has poor electrical conductivity resulting in less sensitivity of biosensors. To overcome this shortage issue, integrating of nanostructured MnO_2 into a 3D porous conductive nanocarbon framework can significantly improve the charge

transfer between MnO_2 and conductive carbon support materials and led to increased electrical conductivity, enhanced electrochemically active surface areas, and improved sensing performance.

Graphene, due to its distinguished electrical, chemical and catalytic performances, has attracted considerable interests in electroanalytical chemistry (Long et al., 2015; Guoqiang et al., 2015; Shenguang, et al., 2015; Lei et al., 2013). 3D graphene foam is regarded as an ideal conductive scaffold with high specific surface area, as well as flexible properties. Cheng and coworkers (Zongping et al., 2011) innovatively synthesized 3D graphene on Ni foam (NF) by chemical vapor deposition, which shows good mechanical and electrical properties. Up to now, the 3D graphene foam manufacturing is generally based on chemical vapor deposition method using nickel foam as the template, which inevitably requires rigid condition and complicated procedure. Therefore, it is still a great challenge to develop a facile, rapid and low-cost method to fabricate high-conductive and mechanical integrated 3D graphene foam under mild conditions (Shuang et al., 2015). It is well known that reduced graphene oxide (RGO) can be obtained by the reduction of graphene oxide (GO) cost-effectively on a large scale, leading to an attractive application prospect for electrochemical devices. Haifu et al (2014) successfully synthesized a novel 3D lightweight graphene composite foam by chemical reduction of GO on Ni foam using hydroiodic acid. While Huijun et al (2015) synthesized 3D graphene foam/ $\text{Ni}(\text{OH})_2$ hybrids for high-performance supercapacitors via a simple electrochemical reduction of GO deposited on Ni foam and followed by a hydrothermal process,

In this paper, we present a facile method to fabricate MnO_2 nanoflowers loaded onto 3D RGO@nickel foam by one-step electrochemical approach. The as-prepared hybrid is denoted as $\text{MnO}_2/\text{RGO@NF}$. As illustrated in Figure 1a, the $\text{MnO}_2/\text{RGO@NF}$ biosensor was fabricated by firstly spraying of graphene oxide (GO) solution on Ni foam to form GO thin-film modified Ni foam (GO@NF), followed with simultaneous electrochemical reduction of GO and electrodeposition of MnO_2 nanoflowers on RGO by one-step electrochemical process. With this strategy, the resulted 3D RGO@NF , employed as both current collector and supporting template without binder for active materials, effectively reduces the connection resistance between the active MnO_2 nanoflowers and RGO@NF . Compared with pure NF as mechanical skeleton, the addition of graphene provides high pathways for electrons. Meanwhile, Ni foam provides better mechanical and flexible properties compared with pure graphene foam. Moreover, the developed electrochemical synthesis protocols avoid high temperature and toxic reduction agents in order to maintain the 3D porous graphene network and possess good electrical connection between MnO_2 and graphene, which facilitates the diffusion of active species and the transport of electrons. Therefore, the as-prepared $\text{MnO}_2/\text{RGO@NF}$ electrode exhibits significant enhancement in electrocatalytic performance for the selective and sensitive detection of ractopamine and salbutamol. Both cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques were used for the simultaneous detection of RAC and SAL in the mixture. The $\text{MnO}_2/\text{RGO@NF}$ biosensor gave a relatively high sensitivity as an amperometric sensor and was also employed for the simultaneous detection of

RAC and SAL in pork samples.

2. Material and methods

2.1 Reagents and apparatus

Ractopamine and salbutamol were purchased from Sigma-Aldrich (USA). The Ni foams ($\sim 320 \text{ g/m}^2$ and $\sim 1.6 \text{ mm}$ thick) were purchased from Shanghai Zhongwei New Material Co. Ltd. All other chemicals were analytical reagent grade and used without further purification. All solutions were prepared with ultrapure water of resistivity $18.2 \text{ M}\Omega\text{cm}$ obtained from a Millipore Milli-Q system.

The crystalline properties and morphologies of the as-prepared materials were characterized by powder X-ray diffraction (XRD, D8-advanced, Bruker, 40 kV, 20mA, Cu $K\alpha$ radiation) and scanning electron microscopy (SEM, JEOL, JSM6700F) equipped with an X-ray energy dispersive spectrometer (EDS). The atomic composition of the samples was detected by X-ray photoelectron spectroscopy (XPS, Perkin Elmer, Al $K\alpha$ radiation). HPLC detection was carried out with an Agilent 1100 coupled with a UV-Vis detector, and the column was C18 analytical column ($4.6 \text{ mm} \times 150 \text{ mm}$, $5 \mu\text{m}$).

2.2 Preparation of $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode

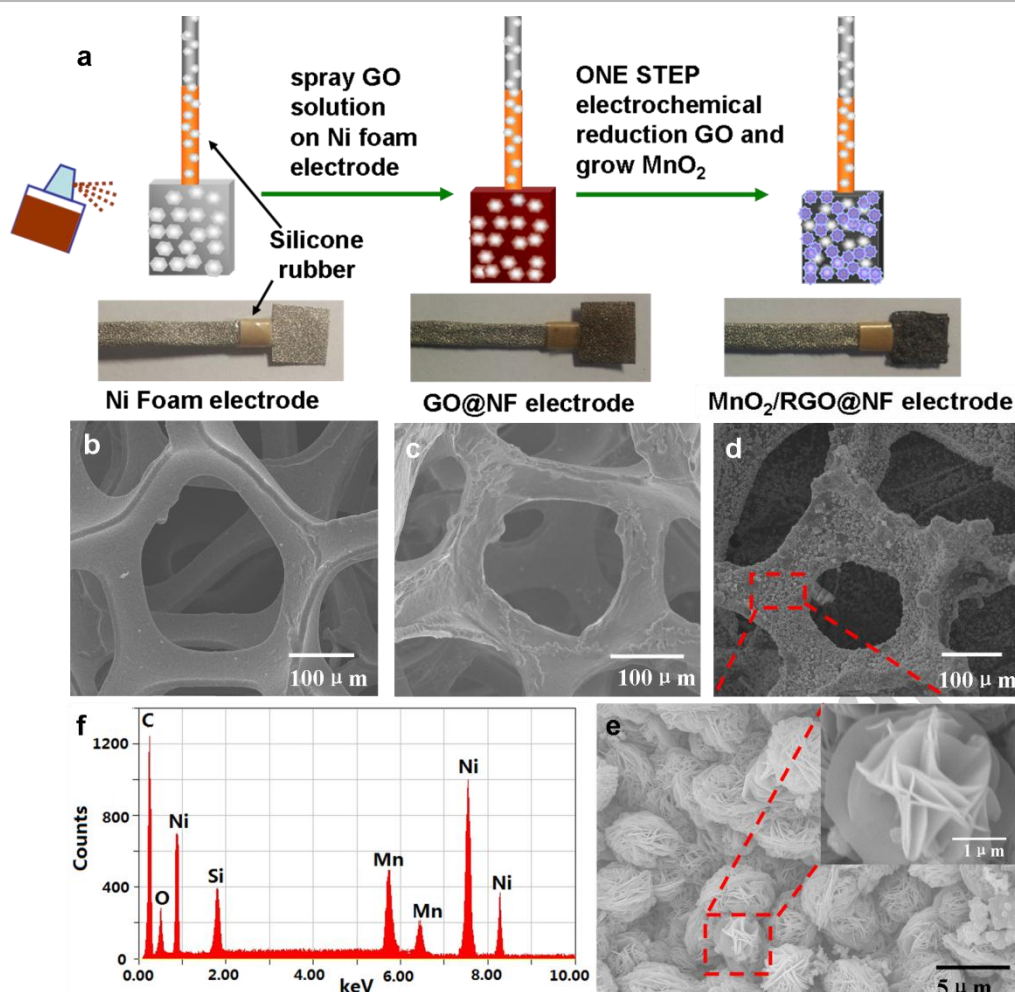


Figure 1 (a) Schematic illustration of preparation of MnO₂/RGO@NF electrode and the photographs of the as-prepared Ni foam, GO@NF, and MnO₂/RGO@NF electrodes; SEM images of Ni foam(b), GO@NF (c) and MnO₂/RGO@NF (d, e) (insert of e: high-resolution SEM image of MnO₂nanoflowers); (f) EDS spectrum of MnO₂/RGO@NF.

The standard biosensor fabrication process is illustrated in Fig. 1a. In order to prepare quality sensing electrode, Ni foam was carefully cleaned and treated with acetone and hydrochloric acid to remove contaminants, and then washed in sequence with ultrapure water and absolute ethanol, before Ni foam is ready for GO deposition. In typically, Ni foam was tailored into a square shape (1×1 cm) with a long handle (0.3×4 cm) as the electrical lead. The electrical active surface of the biosensor was fixed with insulated silicone rubber. Graphene oxide (GO) was synthesized by the

modified Hummers method (Hummers et al., 1958). A certain amount of GO powders were ultrasonically dispersed in ultra pure water for 30 min. The GO solution (from 0.1 to 0.3, 0.5 and up to 0.7 mg/ml) was monitored from a syringe pump (Kd Scientific, KDS100) and fed to a sprayer. The GO spraying process (15 min) was carried out under an air flow (8 L/min) towards the Ni foam which was placed on a hot plate (80 C°) to enhance/promote the solvent evaporation. After spraying, the GO coated Ni foam (GO@NF) was dried in the air overnight. The one-step electrochemical process for both reduction of GO and deposition of MnO₂ nanoflowers were carried out in 0.1 M Na₂SO₄ aqueous solution containing 50 mM Mn(CH₃COO)₂ and 15 mM MnSO₄ by a continuous cyclic voltammetric sweep (30 cycles) from 1.4 to -1.5 V at a scan rate of 25 mV/s. After electrodeposition, the as-prepared hybrid (denoted as MnO₂/RGO@NF) was washed with ultrapure water to remove excessive electrolyte, and further dried at 60 C° in oven overnight. For control comparison, electrochemical reduction of GO on Ni foam and electrochemical deposition of MnO₂ on Ni foam were also prepared under same procedure and identical conditions, which were denoted as RGO@NF and MnO₂@NF, respectively.

2.3 Real sample preparation

According to the reference (Xiaoyun et al., 2013), the real samples were prepared. A finely chopped pork sample (10.0 g) were exactly weighed and spiked with suitable amounts of 332 nM ractopamine or 418 nM salbutamol standard solutions. 20 mL ethyl acetate and 1 mL 4 mol/L K₂CO₃ solutions were added to the pork sample. Then the solution was shaken vigorously by sonication for 60 min. After that the

supernatant was collected via centrifuge and dried at 40 C°. This solid residue was then dissolved in 1.0 mL 50% methanol solution and reconstituted in the buffer solution for electrochemical analysis.

2.4 Electrochemical measurement

Electrochemical measurements were performed using a CHI 660B electrochemical workstation (Austin, USA) with a conventional three-electrode cell. The as-prepared biosensor was used as the working electrode. A platinum wire was used as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode, respectively.

A required volume of each β -agonists sample, mixed with 5.0 mL buffer solution, were transferred to electrochemical cell and diluted to 10.0 mL with ultrapure water. After accumulation for 200 s at open circuit potential, cyclic voltammetry (CV) was performed from -0.1 to 1.4 V with the scan rate of 100 mV/s. The differential pulse voltammetry (DPV) was carried out from 0.25 to 1.20 V with the following parameters: amplitude, 0.05 V; pulse width, 0.05 s; sample width, 0.05 s; pulse period, 0.2 s; quiet time, 2s.

Electrochemical impedance spectra (EIS) measurements were carried out at open potential circuit potential in 0.1 M KCl solution with an ac perturbation of 5 mV in the frequency range from 0.01 Hz to 100 KHz using a solartron SI1260 Impedance Analyzer.

The results were obtained from an average of three parallel experiments. All experiments were carried out at room temperature (25 ± 0.5 C°). High pure nitrogen

was used for deaeration.

2.5 HPLC measurements

The HPLC measurements were performed in triplicate. For determination of ractopamine and salbutamol, the mobile phase consisted of a mixture of methanol and ultrapure water, with a flow rate of 1.0 mL/min. UV detection was performed at 284 nm, and the injection volume was 20 μ L (Guanglong, et al., 2015). Before HPLC analysis, the extracted samples were centrifuged at 4000 rpm for 30 min and the supernatant liquids were filtered using a PTFE syringe filter, 33 mm $\times$ 0.22 μ m.

3. Results and Discussion

3.1 Characterization of MnO₂/RGO@NF electrode

The SEM images of the pure NF, GO@NF and MnO₂/RGO@NF are shown in Fig 1b, c and d, respectively. Compared with pure NF (Fig 1b), Fig 1c clearly shows that the layered GO film has been successfully deposited onto 3D Ni foam surface, with crumpled and scrolled interconnected graphene network (Fig S1). For MnO₂/RGO@NF composite, the 3D graphene skeleton is uniformly covered with MnO₂ particles (Fig 1d). The magnified SEM image (Fig 1e) further demonstrates that the deposited MnO₂ particles are composed of flower-like nanostructures with diameters of about 3-5 μ m. The MnO₂ nanoflower structures consist of multiple layers of MnO₂ nanosheets, which are connected to each other through the center to form 3D flower-like nanostructure (insert of Fig 1e). The EDS elemental mapping analysis suggests the presence of Mn, Ni, C, and O components in the hybrid (Fig 1f). Si signal aroused from the Si substrate.

Typical XRD patterns of GO@NF, RGO@NF, and MnO₂/RGO@NF are presented in Fig 2a. As displayed in Fig 2a (curve 1, 2 and 3), three strong diffraction peaks at $2\theta=44.2, 51.6, 76.1$ correspond to (111), (200) and (220) planes of Ni foam respectively. Except for the peaks deriving from Ni foam, the GO@NF shows a characteristic peak at $2\theta=9.5^\circ$ attributed to the (002) reflection of graphene oxide. The interlayer spacing was much larger than that of pristine graphite because of the introduction of oxygen-containing functional groups on the GO sheets (Xiyan et al, 2011). For RGO@NF (Fig 2a, curve 2), the diffraction peak at 9.5° disappeared after the electrochemical reduction process, and a broad peak at about 23.9° was observed. The shift of the characteristic peak (002) indicates the oxygen containing groups on the graphene sheets was eliminated and GO was successfully reduced by the electrochemical reduction process (Jussi et al, 2013). Besides the characteristic peaks from Ni foam and graphene, the characteristic diffraction peaks of the MnO₂/RGO@NF matched well with the standard peak positions for a crystalline α -MnO₂ (JCPDS Card: 44-0141) (Huajie et al., 2013). This demonstrates the successful preparation of MnO₂/RGO hybrid on Ni foam by a facile method through one-step electrochemical synthesis protocol.

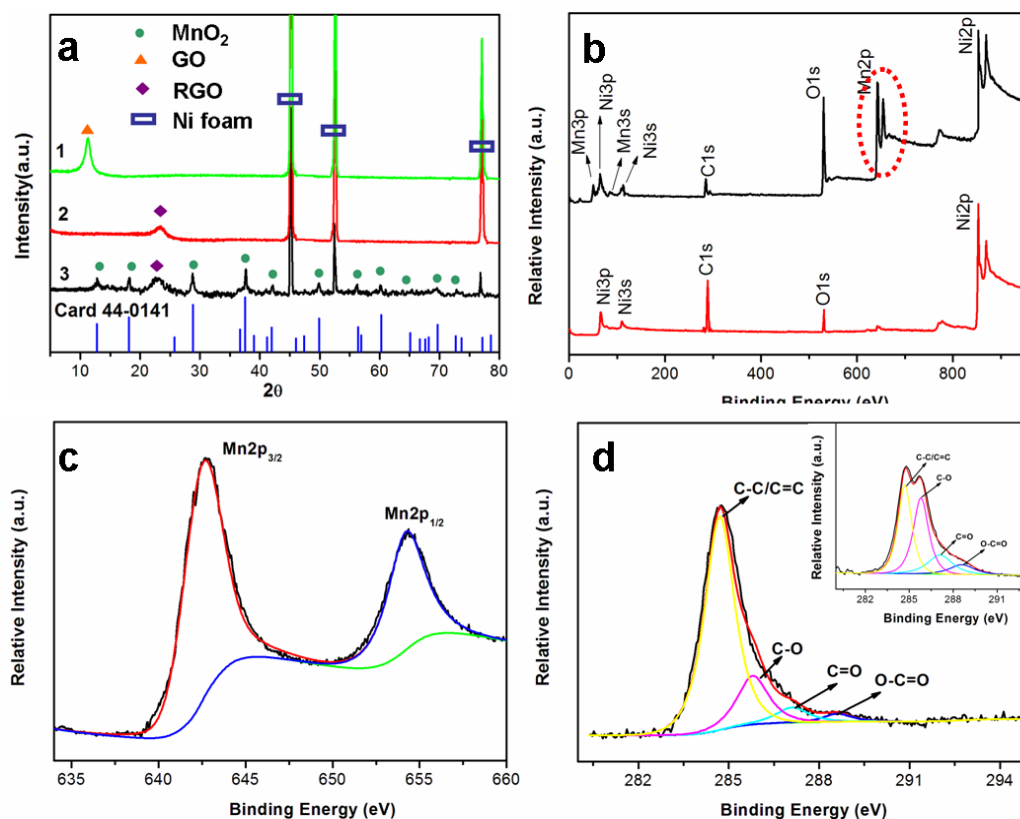


Figure 2 (a) XRD patterns of (1) GO@NF, (2) RGO@NF, and (3) MnO₂/RGO@NF; (b) XPS survey spectra of MnO₂/RGO@NF and GO@NF; (c) Mn2p XPS of MnO₂/RGO@NF; (d) C1s XPS for MnO₂/RGO@NF (Insert of d: C1s XPS for GO@NF).

The XPS wide-scan spectra of the MnO₂/RGO@NF and GO@NF have been shown in Fig 2b. For the sample MnO₂/RGO@NF compared with GO@NF, obvious signals of Mn2p, Mn3p and Mn3s are found at 642.0 eV, 47.0 eV, and 82.0 eV besides the signals of C1s, O1s and Ni2p, indicating MnO₂ successfully deposited on 3D graphene foam (Huijun et al., 2015). The Mn2p XPS spectrum is given in Fig 2c, where the binding energies are at 641.2 eV for Mn_{2p3/2} and 649.8 eV for Mn_{2p1/2}. A spin energy separation of 11.3 eV between Mn_{2p3/2} and Mn_{2p1/2} attributed to formation of MnO₂ in the hybrid (Hongcai et al., 2012).

The C1s XPS spectrum of GO@NF (insert of Fig. 2d) can be deconvoluted into four peaks arising from C-C/C=C (284.6 eV) in the aromatic rings, C-O (286.4 eV)

of epoxy and alkoxy, C=O(287.8 eV), and O–C=O (289.3 eV) groups. For MnO₂/RGO@NF (Fig. 2d), the intensity of the oxygenated groups significantly decreased, indicating that GO was reduced to RGO through the electrochemical reduction (Mingyan et al., 2013). This result is in a good agreement with the results of XRD.

3.2 Electrochemical behavior of ractopamine and salbutamol at MnO₂/RGO@NF electrode

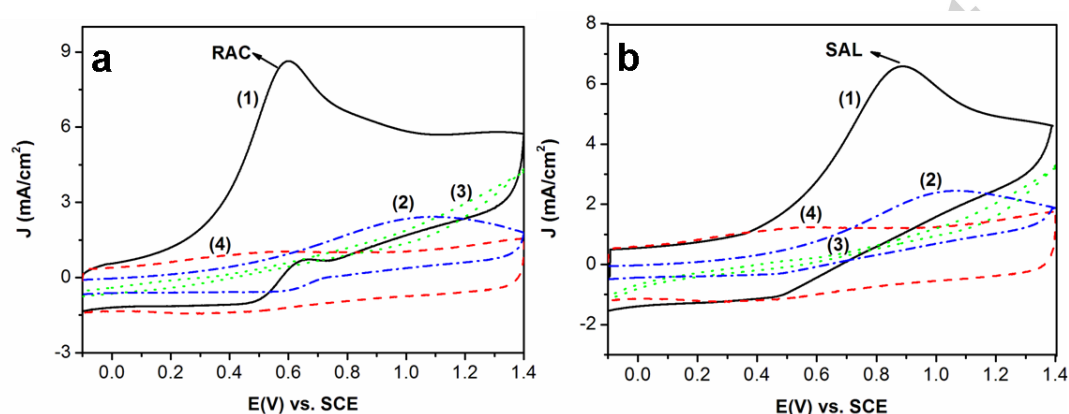


Figure 3 CV curves of 398 nM RAC (a) or 501 nM SAL (b) on MnO₂/RGO@NF (curve 1), RGO@NF (curve 2) and MnO₂@NF (curve 3) electrodes. (Curve 4: CV curve of MnO₂/RGO@NF electrode in the absence of RAC and SAL). Electrolyte solution: 0.2 M pH 6.0 phosphate buffer, N₂ saturated; Scan rate: 100 mV/s.

The electrocatalytic performance of MnO₂/RGO@NF electrode toward RAC and SAL oxidation was investigated in PBS buffer solution (pH 6.0) with a proper comparison with RGO@NF and MnO₂@NF electrodes (Fig. 3). As shown in Fig. 3a, MnO₂/RGO@NF electrode (curve 1) demonstrated a clear catalytic activity to RAC oxidation with an obvious anodic peak appeared after the addition of RAC. The onset potential of RAC electrochemical oxidation using MnO₂/RGO@NF is about 0.2 V, which is much lower than those on both RGO@NF (curve 2) and MnO₂@NF (curve

3), as well as a 7-fold higher catalytic current density, which is critical for detection sensitivity. The similar phenomenon was also found on the $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode (Fig. 3b) for electrocatalytic oxidation of SAL. These consistent observations reveal that the $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode has a superior activity for both RAC and SAL catalytic oxidation.

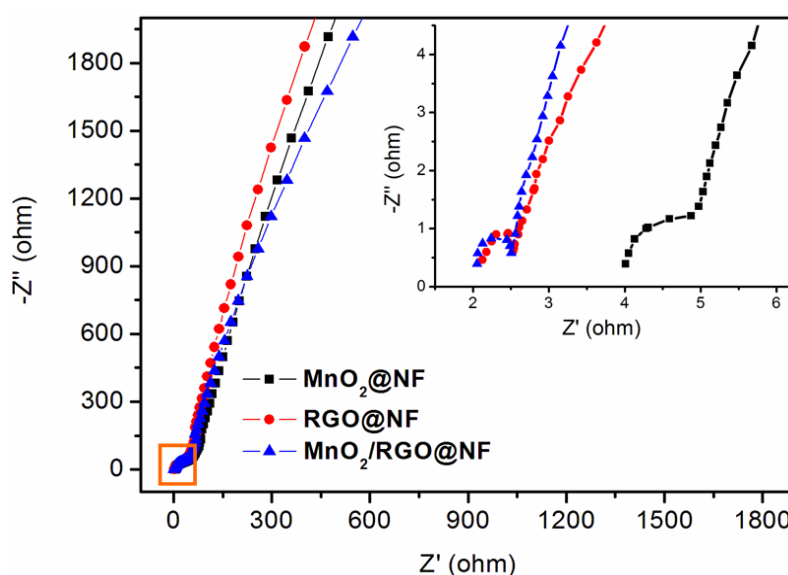


Figure 4 Nyquist plots of $\text{MnO}_2@\text{NF}$, $\text{RGO}@\text{NF}$ and $\text{MnO}_2/\text{RGO}@\text{NF}$ electrodes at open potential. (Insert of Fig. 4: enlarged image of the yellow square in Fig 4.) All measurements were performed in 0.1 M KCl solution containing 0.5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$.

Electrochemical impedance spectroscopy (EIS) was employed to probe the electron transfer kinetics at $\text{MnO}_2@\text{NF}$, $\text{RGO}@\text{NF}$ and $\text{MnO}_2/\text{RGO}@\text{NF}$ electrodes (Fig 4). The semicircle at high frequency of the Nyquist plots corresponds to the charge transfer resistance (R_{ct}) and the linear portion at low frequency is related to capacitance behavior. The intercept of semicircle with the real axis is equivalent series resistance (ESR) containing the resistance of the electrolyte solution, the intrinsic resistance of the active material and the contact resistance of the interface active material and current collector (Qiufan et al., 2013). The ESR and R_{ct} of

$\text{MnO}_2/\text{RGO}@/\text{NF}$ is much smaller than that of $\text{MnO}_2@/\text{NF}$, and almost similar to that of $\text{RGO}@/\text{NF}$. Therefore, with the presence of RGO, it not only decreases the contact resistance of interfaces but also enhances the charge transfer between active materials and conductive supporting template in $\text{MnO}_2/\text{RGO}@/\text{NF}$, which could promote the catalytic RAC and SAL oxidation much effectively and more efficiently than $\text{MnO}_2@/\text{NF}$ without RGO. Thus, the addition of RGO layer plays a key role in the sensitive detection of RAC and SAL, which makes simultaneous determination of RAC and SAL possible.

3.3 Electrocatalytic oxidation of RAC and SAL in a mixture

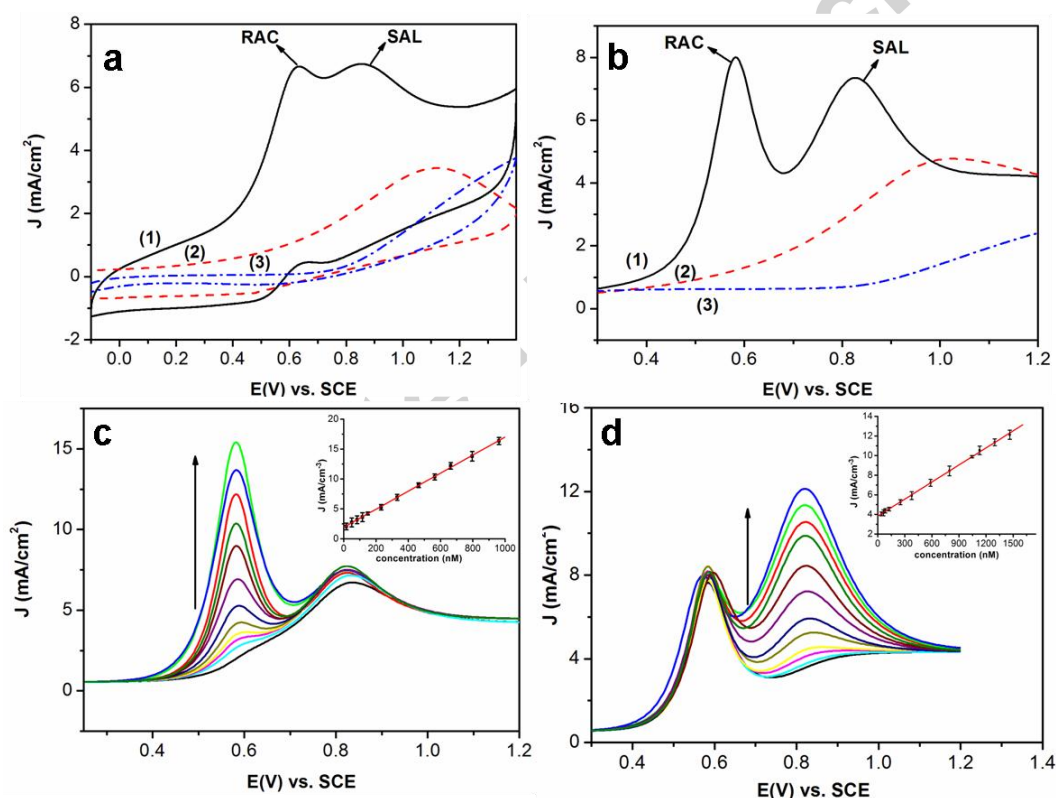


Figure 5 (a) CV and (b) DPV curves of 398 nM RAC and 501 nM SAL in a mixture solution with $\text{MnO}_2/\text{RGO}@/\text{NF}$ (curve 1), $\text{RGO}@/\text{NF}$ (curve 2) and $\text{MnO}_2@/\text{NF}$ (curve 3) electrodes. (c) DPV curves of RAC oxidation with various concentrations 17, 50, 83, 116, 149, 232, 332, 464, 564, 664, 796, 962 nM (from inner to outer) in the presence of 501 nM SAL at $\text{MnO}_2/\text{RGO}@/\text{NF}$ electrode. (Insert of c: plots of electrocatalytic peak currents from (c) versus RAC concentrations.) (d) DPV

curves of SAL oxidation with various concentrations 42, 63, 84, 125, 251, 376, 585, 794, 1045, 1128, 1295, 1463 nM (from inner to outer) in the presence of 398 nM RAC at $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode. (Insert of d: plots of electrocatalytic peak currents from (d) versus SAL concentrations.)

The electrochemical behaviors of $\text{MnO}_2/\text{RGO}@\text{NF}$ in a RAC and SAL mixture were further studied using CV and DPV to investigate the selectivity between RAC and SAL in order to establish a novel detection method with both sensitivity and selectivity for the quantitative determination of RAC and SAL. Fig.5 (a and b) shows the CV and DPV responses of RAC and SAL in a mixed solution at $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode (curve 1) compared with $\text{RGO}@\text{NF}$ (curve 2) and $\text{MnO}_2@\text{NF}$ electrodes (curve 3). The electrochemical response of RAC and SAL were resolved into two well-separated distinct CV peaks at approximately 0.64 and 0.86 V with $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode whereas either $\text{RGO}@\text{NF}$ or $\text{MnO}_2@\text{NF}$ electrode was not able to resolve the peaks, one broad and overlapped peak for both RAC and SAL. In addition, better-resolved peaks were obtained by DPV, i.e., two peaks at 0.58 and 0.83 V for the oxidation of RAC and SAL, respectively (Fig. 5b). The successful selective and sensitive detection of RAC and SAL makes it possible to determine RAC and SAL individually and simultaneously.

3.4 Optimization studies

To optimize the electrochemical analysis, different electrode preparation and electrochemical measurement conditions were assessed to improve the performance of the electrodes (see experimental details in supporting information).

3.4.1 Effect of the electrochemical reduction conditions

The concentration of GO suspension and the cycling number of voltammetric

sweep were optimized to improve the catalytic performance of $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode. From Fig SI 2a, it is observed that with the concentration of GO increasing, the electrochemical signal to RAC and SAL increased at first, which may be attributed to the fact that more graphene generated at the electrode with more effective catalytic sites formed. But when the GO concentration was higher than 0.5 mg/mL, the catalytic current began to fall. The increased concentration of GO might lead to the strong agglomeration and restacking of graphene which could block the active sites and lead to an increased resistance for the mass transport of reactant molecules, thereby impeding catalytic reaction. The optimal GO concentration was 0.5 mg/mL.

The results in Fig SI 2b indicated that through fewer sweep cycles, the electrochemical reduction of GO and electrodeposition of MnO_2 would not be comprehensive enough, resulting in weak catalytic activity to RAC and SAL. However, after over 30 cycles, the deposited MnO_2 nanoflowers would aggregate into larger particles and even fill into the porous structure of Ni foam, which decreased the catalytic activity of the hybrids. In this study, the optimal cyclic number of voltammetric sweep was 30.

3.4.2 Effect of supporting electrolytes and pH

The DPV responses for RAC (398 nM) and SAL (501 nM) with $\text{MnO}_2/\text{RGO}@\text{NF}$ electrode were investigated in different supporting electrolytes. As shown in Fig. SI-3a, the highest peak current was obtained with 0.2 M phosphate buffer as the electrolyte. Therefore, 0.2 M phosphate buffer (pH 6.0) was chosen as the analytical medium, in which the peak shape was well defined. Meanwhile, the

maximum current appeared at pH 6.0 for the determination of both RAC and SAL.

3.4.3 Effect of accumulation

The peak currents of RAC and SAL changed slightly as the accumulation potential changed. This indicates that the accumulation potential had no significant influence on the peak current of RAC and SAL oxidation at MnO₂/RGO@NF electrode. Thus, an open-circuit accumulation was selected for the optimization of accumulation time. The anodic peak current for both RAC and SAL increased gradually as the accumulation time was extended from 10 s to 250 s (see Fig SI4), which can be attributed to the adsorption of RAC and SAL on the electrode surface. Beyond this time frame, the oxidation peak current remained steady. This phenomenon can be attributed to the saturated adsorption of RAC and SAL on the MnO₂/RGO@NF electrode. Thus, 200 s was selected as the accumulation time.

3.5 Calibration and reproducibility

Fig.5c shows the DPV recordings at various RAC concentrations with a constant SAL concentration (501 nM) under the optimized testing conditions at MnO₂/RGO@NF electrode. The peak currents of RAC increased with increasing concentration of RAC in the presence of SAL. The DPV curves clearly indicate that 501 nM SAL has no interference with the determination of RAC within the concentration range of 17 to 962 nM. Similarly, the oxidation peak currents of SAL increased with its increasing concentration from 42 to 1463 nM when the concentration of RAC (398 nM) was kept constant (Fig. 5d). The corresponding regression equation can be expressed as $J_{RAC}(\text{mA}/\text{cm}^2) = 1.92 + 0.015C \text{ (nM)}$

($R=0.9997$) for RAC and $J_{SAL}(\text{mA}/\text{cm}^2) = 3.83+0.006C$ (nM) ($R=0.9999$) for SAL.

The detection limit was calculated as 11.6 nM for RAC and 23.0 nM for SAL ($S/N=3$).

Successive measurements using $\text{MnO}_2/\text{RGO}/\text{NF}$ electrode were examined in a mixed solution. Electrode fouling was not observed after several scans. A relative standard deviation (RSD) of 2.8% was obtained for five successive measurements of RAC (398 nM) and SAL (501 nM). The electrode-to-electrode reproducibility (RSD, $n=5$) for RAC (398 nM) and SAL (501 nM) with freshly prepared modified electrode was determined as 3.5%. The long-term storage stability of $\text{MnO}_2/\text{RGO}/\text{NF}$ electrode was investigated under the storage conditions (exposure to air and ambient temperature). The peak current responses decreased only by 3.4% over the first 7 days and 8.9% for the following month, according to the results obtained from daily measurements of RAC and SAL.

The comparison of the performance of this kind of sensor with other sensors for simultaneous detection RAC or SAL is listed in Table S1 (see supporting information). As shown in the table, the detection limit and linear calibration range of the new sensor are comparable with and even slightly better than those obtained by other hybrid-modified electrodes.

3.6 Analysis of real samples

In order to investigate the durability and selectivity, the $\text{MnO}_2/\text{RGO}/\text{NF}$ electrode was used to detect RAC and SAL in real pork samples. As shown in Table 1, all samples were either contaminated with concentrations below the detection limit or

absolutely free of RAC and SAL. A recovery study was carried out with the sensors using the standard addition method and direct interpolation in the linear regression. The determined values were in agreement with the assigned value for each substrate in the samples. The satisfactory recoveries (96.2% to 104.2%) of MnO₂/RGO@NF electrode for RAC and SAL detection in pork samples confirm that this electrode is a stable and sensitive sensor for analyzing real samples.

To evaluate the accuracy of the biosensor the concentration of RAC and SAL were also analyzed by HPLC. The HPLC chromatogram of RAC (spiked at 33.2 nM) and SAL (spiked at 62.7 nM) in a pork sample is shown in Fig. SI-5. The analysis of statistically significant difference of the two techniques showed that the results obtained with this sensor were in satisfactory agreement with data from the reference method obtained at 95% confidence level using the paired t-test model. Supported by this HPLC control detection tests results, the accuracy of the biosensor performance is comparable with HPLC, which presents this sensor as an attractive candidate for practical application in real samples.

Table 1 Under the optimized condition simultaneous determination of RAC and SAL in pork samples by MnO₂/RGO@NF electrode and HPLC.

Samples	Analyst	Added (nM)	By this method ^a (nM)	By HPLC ^a (nM)	Rel error (%)	Recovery (%)	RSD (%)
1#	RAC	33.2	32.1	33.5	-4.26	96.6	3.1
	SAL	62.7	64.1	63.3	1.32	102.3	3.9
2#	RAC	99.5	95.8	93.9	1.94	96.2	3.4
	SAL	208.9	216.0	226.7	-4.75	103.4	2.2

3#	RAC	165.9	172.9	170.3	1.54	104.2	4.3
	SAL	417.9	407.6	419.4	-2.82	97.5	3.6

^a Each value is the average of three determination.

The selectivity of the sensor is mandatory for practical applications. So the selectivity of the sensor was evaluated by DPV under the above optimized conditions in presence of various possible interfering substances. The following species did not interfere with the oxidation signal of RAC and SAL (i.e., signal change below 10%): 50-fold of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , Al^{3+} , F^- , Cl^- , CO_3^{2-} , SO_4^{2-} , and NO_3^- ; 10-fold of glucose, tyrosine and hydrogen peroxide; and 8-fold of ascorbic acid, uric acid, phenol, catechol, and caffeine. Therefore, this novel $\text{MnO}_2/\text{RGO}@\text{NF}$ sensor provides an excellent selectivity for RAC and SAL.

4. Conclusions

3D $\text{MnO}_2/\text{RGO}@\text{NF}$ hybrid was synthesized and developed to fabricate a novel freestanding biosensor. The as-synthesized $\text{MnO}_2/\text{RGO}@\text{NF}$ sensor not only displayed an excellent electrocatalytic activity (both sensitivity and selectivity) toward RAC and SAL oxidation, but also showed high linear relation and accuracy for simultaneous detection of RAC and SAL in pork samples. The simple fabrication procedure, wide linear range, low detection limit, high stability and well selectivity and accuracy make this sensor has the potential to be developed and used in environmental and biological analysis for RAC and SAL.

Acknowledgements

This work was supported by National Natural Science Foundation of China (51202079 and 21201070), Natural Science Fund of Jiangsu Province (BK20141247,

BK20140447), University Science Research Project of Jiangsu Province (13KJB430005, 11KJA430008). A project funded by the Priority Academic Program development of Jiangsu Higher Education Institutions. The authors are grateful to Jiangsu Province universities' "blue and green blue project". The authors would also like to thank the Australian National Fabrication Facility (ANFF) – Materials node for equipment access.

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HIGH LIGHT

- A facile method to successfully fabricate MnO_2 nanoflowers loaded onto 3D RGO@nickel foam was developed, the as-prepared 3D hybrid electrodes were investigated as a binder-free biosensor.
- The novel 3D biosensor exhibited excellent electrocatalytic performance for ractopamine (RAC) and salbutamol (SAL).
- The developed $\text{MnO}_2/\text{RGO}/\text{NF}$ sensor was further investigated to detect RAC and SAL in pork samples, showing satisfied comparable results in comparison with analytic results from HPLC.