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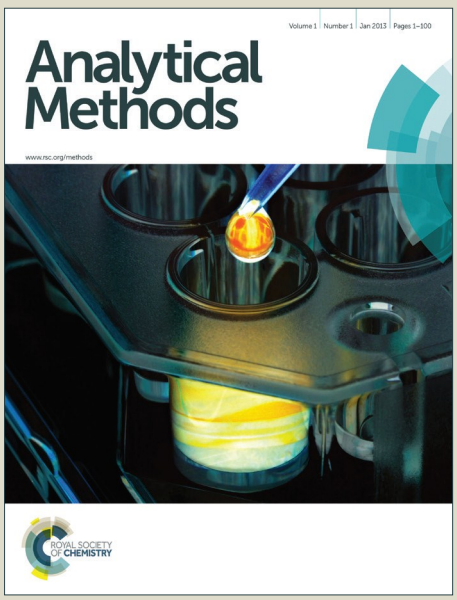

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ARTICLE

One-piece lateral flow impedimetric test strip for label-free clenbuterol detection

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ZhuanZhuanShi,[abc] YunLi Tian,[abc] XiaoShuai Wu,[abc] ChangMing Li [abc] and Ling Yu*[abc]

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A one-piece lateral flow impedance strip was developed for detection of clenbuterol hydrochloride, a restricted food additive. The immunochromtograph strip was sandwiched between microelectrodes patterned on a thin poly(ethylene terephthalate) (PET) film. To demonstrate the label free assay capability of the electrode/lateral flow impedance strip, polyaniline@graphene oxide (PANI@GO) nanocomposite was synthesized to functionalize the sensing electrode. The morphology, chemical functional group and electrochemical stability of synthesized polyaniline/graphene oxide nanocomposite were characterized by field emission scan electron microscopy (FESEM), fourier transforms infrared (FTIR), and electrochemical impedance spectroscopic (EIS). The fully assembled one-piece lateral flow impedance strips were successfully applied for detection of clenbuterol hydrochloride (CLH) with a working range of 0.12-58 ppb and a lower detection limit of 0.12 ppb, demonstrating a universal method to label-free, sensitive detection of small target molecules by a portable strip for applications in food safety.

1. Introduction

Clenbuterol hydrochloride (CLH) is a β-agonist that has been used in the treatment of human depression and pulmonary diseases[1]. Unfortunately, CLH has also been used illegally by farmers to make their pigs leaner[2, 3]. The long half-life and stability of CLH residues in livestock raise a potential risk for human health[4]. Thus, administration of CLH to any animal that could be used as food for human consumption is banned the Food and Drug Administrations from many countries. To supervise CLH usage, different analytical approaches have been applied for the identification of CLH. High-end techniques such as gas chromatography–mass spectrometry (GC–MS)[5], high-performance liquid chromatography (HPLC)[6-8], nuclear magnetic resonance spectrometry (NMR), capillary electrophoresis (CE)[9, 10], and enzyme-linked immunosorbent assay (ELISA)[11] have been used to analyze CLH. Even though these assays have demonstrated high sensitivity, they have limited applications in-field testing, especially for rural countries that do not have access to equipment for distant services. Thus, a simple, portable, disposable, and quantitative CLH detection tool is highly desired to fulfill daily food safety supervision, especially for under-developed regions.

There are tremendous requirements for point-of-care (POC) detection of pathogens and biohazards that drive market efforts being paid on invention of low-cost, user friendly, and accurate biosensors. Lateral flow strips based on the principle of immunochromatographic have been recognized as an economical diagnosis tool in detecting chemical and biochemical molecules in various applications[12-14]. These lateral flow strips (LFS) allow a one-step, rapid and inexpensive analysis by end users[15-17]. Most recently, Du et al. incorporated an electrochemical sensor coupled to a flow strip to innovate the detection scheme of lateral flow strip; except in their study, the testing zone/sensing pad is to be cut from the strip and placed in an electrochemical cell which is located under the test zone; this step might diminish the simplicity of operation. In addition, it is important to point out that the mainstream of lateral flow strips heavily involves a signal reporter that is conjugated with a biological probe to characterize the reaction. Although the labeling detection scheme increases the assay sensitivity, it potentially destroys the protein function during labeling process and increases the cost of the test. To achieve a low-cost, user friendly assay scheme, label-free detection is highly desired[18].

Electrochemical impedance biosensors are a class of label-free sensors that show promise for point-of-care and other applications. Chen et al. reported an impedance sensor for biomedical protein IL-5 detection with a sensitivity of 1 pg/mL in 1% human serum[19]. While Wang et al. assayed small molecule aflatoxin B1 using label-free impedance sensors[20]. Therefore, the sensing materials are of critical importance for constructing a sensitive impedance biosensor, Polyaniline (PANI) is attractive due to its simple synthesis, low cost, high conductivity, and excellent environmental stability[21, 22]. However, degradation of PANI during electrochemical processes (doping and de-doping) challenges its applications in biosensing and capacitors[23, 24]. For instance, to achieve improved electrochemical performance as a super capacitor electrode, graphene-reinforced PANI composites were synthesized with different strategies, such as chemical oxidation synthesis and electrochemical synthesis[25]. Specifically, electrochemical synthesis approaches include cyclic voltammetry (CV), galvanostatic, potentiostatic and

pulse current methods. However, there has been little investigation of PANI/graphene nanocomposites as a sensing material for an impedance biosensor.

To fulfill a low-cost and daily-food supervision aim, we report a one-piece lateral flow impedance strip for the in situ sensing of CLH. A competitive immunoassay reaction scheme was demonstrated on a paper-based lateral flow strip that was sandwiched between indium tin oxide (ITO) electrodes patterned on thin PET film. To achieve sensitive CLH detection, a PANI@GO nanocomposite was electrochemically deposited on the ITO, functioning as a working electrode (WE). The trace amounts of CLH in the testing sample competed with ovalbumin-conjugated CLH (OVA-CLH) that was covalently immobilized on the WE to bind with anti-CLH monoclonal antibody (mAb). The binding events between OVA-CLH and anti-CLH mAb were monitored by electrochemical impedance spectroscopic (EIS) measurements. The synthesized condition of PANI@GO was optimized by analyzing the morphology and electrochemical activity and stability of the composite. The sensitivity and detection working range of the fully assembled strip was characterized, highlighting the considerable potentials for unequivocal diagnosis of food poisoning on-site.

2. Materials and methods

2.1. Materials

Graphite, clenbuterol hydrochloride (CLH), anti-clenbuterol monoclonal antibody (anti-CLH mAb), ovalbumin (OVA), Cy3-labeled anti-rabbit IgG antibody, rabbit IgG, sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH) and glutaraldehyde solution were all purchased from Sigma Aldrich. Aniline, perchloric acid ($HClO_4$), sodium chloride (NaCl), 30% hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phosphate buffered saline (PBS), potassium permanganate ($KMnO_4$) were from Chongqing Chemical Co. (Chongqing, China). Indium tin oxide (ITO)-coated polyethylene terephthalate (PET) film (conductivity:10 $\Omega \cdot cm^{-2}$) was purchased from Shenzhen Semiconductor Co. (Shenzhen, China). Dialysis bag (cut off size: 8000-14000D) were received from scientific research special (USA). The sample pad, adsorbent pad (SX27) and polyvinyl chloride (PVC) backing card were purchased from Shanghai Kinbio Tech (Shanghai, China). All chemicals were used without further purification unless otherwise indicated. All solution was prepared with deionized (DI) water produced by PURELAB flex system, ELGA Corporation.

2.2. One-step electrochemical synthesis of PANI@GO nanocomposite film

Graphene oxide (GO) was prepared from graphite powder by using a modified Hummers method[26]. ITO electrodes were fabricated by using standard photolithography patterning and subsequent etching. The ITO work electrode (WE, 3 mm×3 mm) was coated with PANI@GO film by the following electrochemical deposition procedure: 1) placing electrodes in 1 M $HClO_4$ solution containing 0.3 M aniline and 0.4 mg/mL GO; 2) the polymerization was conducted with a constant current deposition with a current density of 0.6 $mA \cdot cm^{-2}$ for 3 min, then a current density 0.3 $mA \cdot cm^{-2}$ for 15 min; 3) after polymerization, the electrode was washed in DI water carefully and dried at room temperature. The resulting electrode was denoted as PANI@GO/ITO and used for assembling of the one-piece lateral flow strip.

2.3. Characterization of PANI@GO nanocomposite

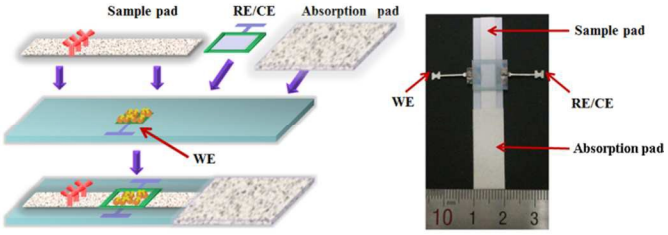
Morphology: Field emission scan electron microscope (FESEM, JSM-7800F) was utilized to examine the morphology of electrochemical synthesized PANI@GO nanocomposite film on ITO electrode.

Fourier transforms infrared (FTIR) spectra: FTIR spectra of the PANI and PANI@GO electrodes were recorded by a FTIR spectrophotometer (Thermo-Nicolet 6700, Japan) with air as a reference. A resolution of 4 cm^{-1} and 32 scans were applied.

Electrochemical stability: A three-electrode system consisting of a PANI@GO/ITO working electrode, an Ag/AgCl reference electrode and a platinum plate counter electrode were employed to characterize the electrochemical properties of the PANI@GO nanocomposite with a CHI760E electrochemical workstation (Chen Hua Instruments Co. Ltd). The electrochemical activities of the composite were studied by CV at 50 mV/s in 1 M NaCl, the potential range was -0.2 to + 1.2V. EIS was performed at the apparent formal potential 0.25 V (E0') extrapolated from voltammograms. To investigate the electrochemical stability of the PANI@GO, the EIS measurement was continually scanned up to 60 min.

2.4. Assemble of lateral flow impedimetric strip

The lateral flow impedimetric test strip consists of a sample loading pad, adsorbent pad, backing card, and two thin film electrodes as illustrated in Scheme 1. The sample loading pad (30mm×4mm) was made of glass fiber paper. First, the PANI@GO/ITO WE was assembled on an adhesive plastic backing card. Next, the sample pad was assembled over WE and then overlapped with the adsorbent pad (20 mm×8 mm) around 2 mm to ensure the solution migrating through the entire strip during assay. Finally, the strip was assembled with a reference/counter electrode (RE/CE) that was opposite the work electrode.



Scheme.1 Schematic illustration of the assembling of one-piece lateral flow impedentric test strip. WE: working electrode, RE: reference electrode, CE: counter electrode.

2.5. On-strip label free competitive immunoassay for clenbuterol hydrochloride detection

Immobilization of ovalbumin-conjugated CLH (OVA-CLH) on PANI@GO nanocomposite film: The OVA-CLH was immobilized on the electrode utilizing the amino-group on PANI as an anchor. First, 5 μL 0.01 mol/L PBS containing 2.5% glutaraldehyde (v/v) was drop casted onto the PANI@GO/ITO working electrode and incubated for 1.5 h at room temperature (RT), resulting in glutaraldehyde-modified PANI@GO films. Then, 5 μL of OVA-CLH solution (1 mg/mL) was added onto the glutaraldehyde-modified electrode and incubated at 37℃ for 2h. The excess OVA-CLH was removed by washing the electrode with PBS 3 times. Finally, the PANI@GO/ITO working electrode immobilized with

OVA-CLH was assembled on the strip as illustrated in Scheme 1.

One-step detections of CLH on lateral flow impedimetric strip: The EIS measurements were conducted to measure the impedance value before and after applying testing samples containing different amounts of CLH. In brief, after conducting the first run EIS (1st EIS) measurement, a 15 μL sample was drop casted onto the sample pad and placed at RT for 30 min. The solution was migrated through the anti-CLH mAb coated antibody region due to the capillary force and reached the testing zone which is tightly sandwiched between the OVA-CLH coated WE and CE/RE. Next, 30 μL of 1M NaCl was drop wise casted onto the sample pad and wet the whole strip. Then, the second run of EIS (2nd EIS) measurement was conducted.

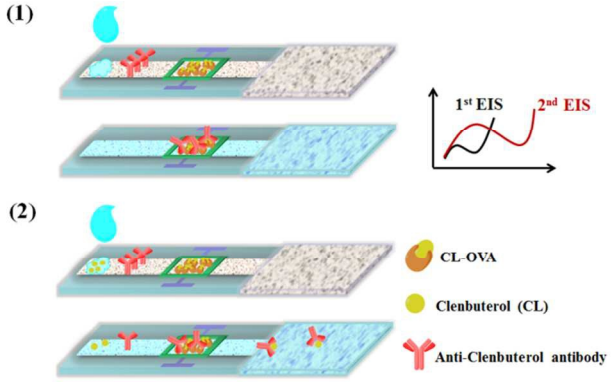
2.6. Statistical analysis

Results were analyzed with the Student's t-test using an Origin Statistic software (OriginLab Corporation, USA). A p-value < 0.05 was considered to be statistically significant. All experiments were performed three times in triplicates independently.

3. Results and discussion

3.1. Assay strategy of one-piece lateral flow impedimetric test strip for clenbuterol hydrochloride detection

Scheme 2 illustrates the measuring principle of the label-free lateral flow impedimetric strip. The sample pad, working electrode and adsorption pad is laminated on an adhesive backboard. The face-to-face assembled WE and RE/CE builds up an electrochemical module. CLH in the testing sample migrated on paper-based sample pad and the OVA-CLH coated probes on the WE are expected to competitively react with anti-CLH mAb. The bonding event between anti-CLH mAb and OVA-CLH probe on WE can be recorded through EIS measurement.



Scheme.2 Schematic illustration of on-strip label free competitive immunoassay for clenbuterol hydrochloride detection. (1) Aqueous sample without CLH: anti-CLH mAb immobilized on strip will migrate to the OVA-CLH functionalized working electrode (WE); the binding event of anti-CLH mAb and OVA-CLH will be quantified by an increased EIS signal; (2) Aqueous sample with CLH: CLH will reacted with anti-CLH mAb pre-coated on strip, thus less anti-CLH mAb can migrate to WE and react with OVA-CLH. The amount of CLH in sample can be quantified by a minimized EIS signal change.

To demonstrate the assay principle, probe (rabbit IgG, 100 μg/mL) was arrayed on a glass slide and a sample pad was laminated over the arrayed-slide. The Cy3-labeled anti-rabbit IgG mAb (1 μg/mL) was pipetted onto one-end of the sample pad. The bonding events between rabbit IgG and Cy3-labeled anti-rabbit IgG mAb were quantified by measuring the fluorescent intensity from the arrayed-slide. As shown in Fig. 1, Cy3-labeled anti-rabbit IgG mAb can migrate along the sampler pad and react with rabbit IgG that was immobilized on a surface under the sample pad. Nonspecific absorption is nearly avoided because no fluorescent signal can be observed from the non-paired array and washing steps do not reduce the signal intensity. Thus, this assembling strategy was applied to construct the one-piece lateral flow impedimetric strip.

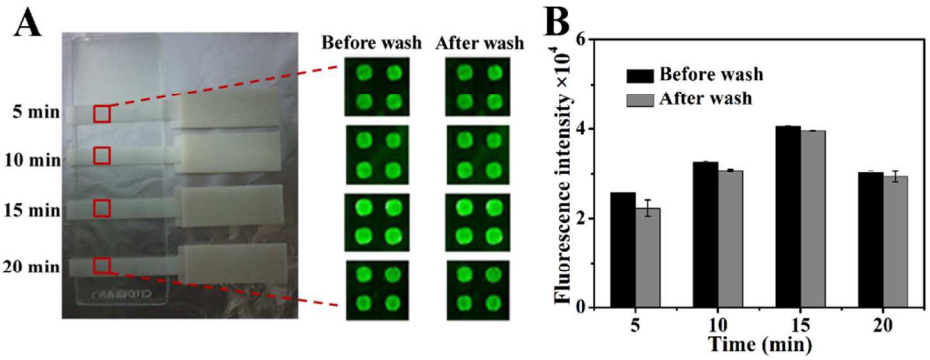


Fig.1 (A) Images of fluorescent labeled antibody characterized antibody-antigen reaction: rabbit IgG was printed on epoxy group functionalized glass slides and a sample pad was laminated over the arrayed-slide. Cy3-labeled anti-rabbit IgG was dropped on one end of strip and allowed for migrating on strip for 5, 10, 15, and 20 min. Then the fluorescent signal on the arrayed glass was measured before and after wash. (B) Histogram of fluorescent intensity of arrayed spots from three independent assays.

3.2. One-step synthesized PANI@GO nanocompiste with improved electrochemical stability

According to the literature, PANI@GO composites can be synthesized by step-by-step dip-coating of PANI and GO[27]. In our study, to simplify the preparation process, a one-step co-deposition of PANI and GO approach was explored to prepare the PANI@GO film. First, to evaluate the electrochemical properties of the PANI@GO nanocomposite, the CV behavior of PANI and PANI@GO films on the ITO electrode were characterized at 50 mV/s in 1 M NaCl (Fig. 2). The redox peaks (A/A', B/B') refer the electroactivity of the PANI-based material[28] can be observed from the PANI film and PANI@GO composition. It is important to note that PANI@GO nanocomposite possess a higher peak current of the fingerprint peak of aniline compared to PANI electrodes, indicating an

improved electrochemical activity and larger electrochemical active area.

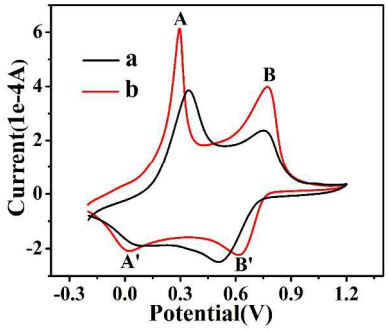


Fig.2 The cyclic voltammograms of PANI (a) and PANI@GO nanocomposite (b) scanning in 1 M NaCl with a scan rate of 50 mV/s.

To find the optimized conditions for synthesis of PANI@GO with improved electrochemical stability, different concentrations of GO and electrochemical polymerization times were investigated. The electrochemical stabilities of PANI and PANI@GO films were compared by continually scanning EIS up to 100 min at RT. The electronic equivalent circuit top inset of Fig. 3A was used to analysis the impedance behaviour of the modified electrodes[29,30]. The impedance spectra measured at 0 and 100 min were compared (Fig.3A right inset). To quantify the stability of the sensing material and eliminate the variation rooted in electrodes, the changes of R is normalized according a previous report[30]:

$$\Delta R = (R_2 - R_1)/R_1$$

where R_1 and R_2 are the resistances measured at the beginning and an immersed time, respectively. Good stability of the nanocomposite were represented by a small ΔR[19]. The results show that ΔR of PANI@GO synthesized from a mixture containing 0.3 M aniline and 0.4 mg/mL GO is characterized by a ΔR-time curve with the smallest slope (Fig. 3A). Fig. 3B shows the histogram of average ΔR's of three independent electrodes scanned at 60 min. During the same period of time, the ΔR of a pure PANI film is nearly 0.263, and the value obtained from PANI@GO synthesized from a mixture containing 0.2 mg/mL, 0.4 mg/mL, and 0.6 mg/mL GO for 15 min is 0.080, 0.024, and 0.155, respectively. Shortened or prolonged deposition times reduce the stability of the composition. The most stable sensing materials are PANI@GO synthesized with a mixture containing 0.3 M aniline and 0.4 mg/mL GO for 15 min.

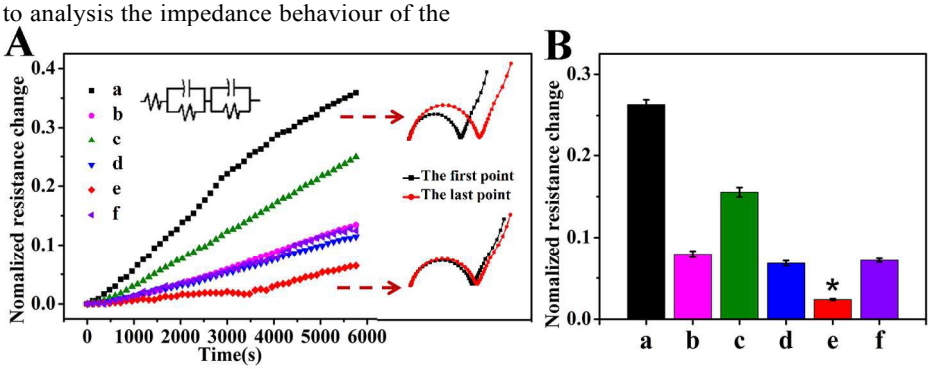


Fig.3 Comparison of PANI and PANI@GO nanocomposites: A. Electrochemical stability comparison of the PANI and the PANI@GO nanocomposites films in 1M NaCl at room temperature. *Right inset.* The comparison of EIS curves at 0 min and 100min, of which PANI and the PANI@GO nanocomposites synthesized under conditions ***a*** and ***e***. *Top inset.* The equivalent circuit used for analysis of impedance data in this study. B. After 1h the electrochemical stability of different nanocomposites films: a. PANI 3,15min b. PANI@GO (0.2mg/ml) 3, 15min c. PANI@GO (0.6mg/ml) 3, 15min d. PANI@GO (0.4mg/ml) 3, 10min e. PANI@GO (0.4mg/ml) 3, 15min f. PANI@GO (0.4mg/ml) 3, 20min (* denotes $p<0.05$, n=3).

To understand the mechanism of improved electrochemical stability, the morphology of nanocomposites synthesized under different conditions was compared. Fig. 4 shows FESEM images of PANI and PANI@GO nanocomposite films. Without incorporation of GO, constant current electrochemical deposition leads to a PANI network dominated by nanowires with a diameter of 100 nm (Fig. 4A). The PANI@GO nanocomposites synthesized from PANI mixed with GO are characterized by GO sheets occupying space between PANI nanowires. Importantly, stacking and aggregation of GO was observed from nanocompiste synthesized from a mixture containing 0.4 mg/ml GO (Fig. 4E). The aggregation phenomena were also observed from composites with shorter or longer deposition times (Fig. 4D and F). This leads us to believe that GO sheets and PANI aromatic rings can stack on top of each other due to the π-π stacking during the deposition process. The GO sheet is believed to reinforce the nanowire network and enhance the physical stability of the nanostructure, therefore potentially minimizing the doping-dedoping process induced structure changes. The above electrochemical impedance data (Fig. 3) prove that the incorporation of GO significantly enhances the stability of the PANI composite.

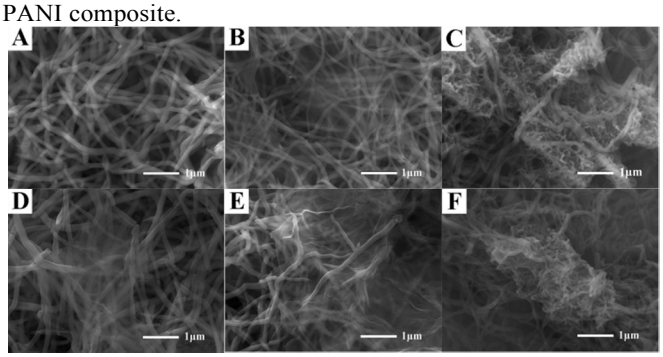


Fig.4 The FESEM of PANI and PANI@GO nanocomposites: A. PANI 3,15min; B. PANI@GO (0.2mg/ml) 3, 15min; C. PANI@GO (0.6mg/ml) 3, 15min; D. PANI@GO (0.4mg/ml) 3, 10min; E. PANI@GO (0.4mg/ml) 3, 15min; F. PANI@GO (0.4mg/ml) 3, 20min.

Next, FTIR spectroscopy data was obtained to characterize the chemical functional groups on the PANI@GO nanocomposite (supplementary data sFig. 1). Compared to the characteristic peaks positioned at 753 and 898 cm^{-1}, 1138 cm^{-1},

2355 and 2881 cm^{-1} to 3300 cm^{-1} which corresponds to the aromatic ring, C–N stretching in aromatic amines, –N-N in diazonium salts, N-H stretching and free O–H stretching vibration of pure PANI, respectively, several new peaks attributed to GO appeared in the PANI@GO spectrum. The C–O stretching of the alkoxy group (1040 cm^{-1}) and O–H stretch peak (3226 cm^{-1}) prove that several types of functional groups exist in GO.

In summary, one-step electrochemical deposition of PANI@GO nanocomposite was successfully conducted. Reaction mixtures containing 0.3M aniline and 0.4 mg/mL were chosen to prepare working electrodes for assembling of the lateral flow impedimetric strips.

3.3. Impedimetric strip based on PANI@GO nanocomposite for one-step label-free detecting clenbuterol hydrochloride

With the synthesized PANI@GO nanocomposite, a face-to-face arranged two-electrode system is applied to construct the one-piece lateral flow impedimetric strip to achieve one-step label-free competitive immunoassay (Scheme 2). The trace amounts of CLH in the testing sample and the OVA-CLH immobilized on electrode compete to capture the anti-CLH mAb. In a competitive immunoassay scheme, the amount of probe immobilized on the working electrode (WE) and antibody on the strip directly affects the assay sensitivity. Therefore, a direct immunoassay was performed by coating OVA-CLH on the electrode and then measuring of different concentrations of anti-CLH to find the lowest anti-CLH amount that can consume all OVA-CLH probes on the electrode. As shown in sFig. 2, a linear signal-dose curve was obtained from the impedance sensor. The plateau was 10 ng/mL of anti-CLH mAb. Thus, to construct the fully assembled device for CLH detection, the OVA-CLH (1 mg/mL) was covalently immobilized on the PANI@GO nanocomposite modified electrode, while anti-CLH mAb (10 ng/mL) was placed on the strip. The analytical performance of the fully assembled one-piece impedimetric strip was characterized with samples containing different concentration of CLH. ZSimpwin (EChem Software) was used to analysis the EIS data. Fig. 5A inset displays the original EIS curve and equivalent circuit simulated curve. It is found that the applied equivalent circuit fit the impedance spectra faithfully. In addition, the impedance spectra obtained from fully assembled strip is not dominated Warburg impedance but resistance signal[31]. Fig. 5A displays the EIS of different concentration of CLH. The blank EIS corresponds to the impedance before sample assaying. Higher concentration of CLH in the assay sample results in a smaller impedance value. The calibration curve of ΔR *vs.* different concentrations of CLH (Fig.5B) demonstrates a linear relationship over a dynamic range of 0.12-58 ppb ($\Delta R = 2.7013\text{-}1.503 \lg[C_{CLH}]$, $R^2=0.993$) with a detection limit of 0.12 ppb (n=3). The analytical performance of the fully assembled strip satisfies most of the critical application requirements. Comparing the existing analytical methods for CLH detection (Table1), our one-piece impedimetric strip achieves a disposable, label free and sensitive detection. We believe the immunochromtograph inspired architecture of the strip contributes to a disposable and fast detection. The one-step co-deposition synthesized PANI@GO proves sensitive label free assay comparing to previous reported PANI-based biosensor (supplementary data Table 1). The significant improvement in sensitivity can be attributed to the improved electrochemical stability, excellent conductivity, and effective covalent probe-immobilization ability of the PANI@GO composite sensing layer. Most encouraging is that the flexible strip can be a low-cost portable candidate for real-time monitoring of small molecules like CLH.

Table 1 Comparison of different CLH detection methods.

Detection method	Analytical performance		Detection platform			Ref
	linear range ng mL^{-1}	Detection limit ng mL^{-1}	Electrode	Label-free	Disposable strip	
EIS	**0.12-58**	**0.12**	**ITO**	**YES**	**YES**	**a**
ECL	0.05-1000	0.02	GCE	-	NO	32
DPV	0.8-1000	0.32	GCE	YES	NO	33
Amperometric	0.5-10	0.2	GCE	NO	NO	34
DPV	0.01-10	0.0068	GCE	NO	NO	35
DPV	0.5-1000	0.25	GCE	NO	NO	36
Amperometric	0.1-10	0.1	SPE	NO	NO	37
SPR	2-20	2	Au	-	NO	38
MS/MS	1-1000	1	-	-	NO	39
MIR and Raman spectroscopy	5-10000	5	-	-	NO	40

a This work; ITO: indium tin oxide; GCE: glass carbon electrode; SPE: screen printed electrode; Au: gold; EIS: electrochemical impedance spectroscopy; ECL: electrochemiluminescence; DPV: differential pulse voltammetry; SPR: surface plasmon resonance; MS: mass spectrometry; MIR: mid infrared.

Storage stability of the strips was evaluated at 25℃ and -20℃ with or without N_2 protection. The results in Fig. 5C show that the strip is more stable when stored at -20℃ with N_2 protected as compared to other conditions. The total increase in stability at 8 weeks was found to be around 4.6%.

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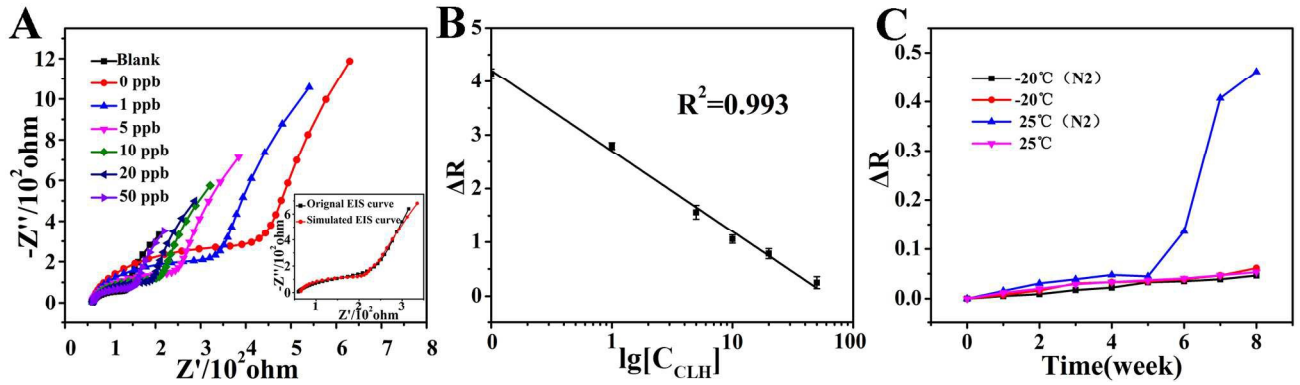

Fig.5 Performance of the one-piece lateral flow impedimetric strip for label free detection of CLH: (A) On-strip competitive immunoassay analyzed different concentration of CLH by EIS; *Inset.* The comparison of original EIS curve and its simulated curve; (B) calibration curve of CLH concentration vs normalized EIS signal change (ΔR), n=3; (C) stability of the OVA-CLH functionalized lateral flow impedimetric strip.

3.4 Determination of clenbuterol hydrochloride in pork juice

To explore the feasibility of the label-free lateral flow impedimetric strip for meat safety inspection, the strip was applied to detect CLH-spiked pork juice. Since there are a large amount of macromolecule, such as protein and lipid in pork juice, it is important to pretreat the sample before detection. The smashed pork was centrifuged (8000 rpm for 10 min at 4°C) to reduce unexpected interference from various components and additives. Then different concentrations of CLH were spiked into 50% centrifuged pork juice. Fig. 6A shows the EIS curves measured with pork juices spiked with different concentration of CLH. Because the spiked CLH consumed anti-CLH mAb on the strip, less antibody will react with the immobilized OVA-CLH which is characterized by a smaller impedance value. The dose-signal calibration displays a linear relationship between ΔR and concentrations of CLH in pork juice (Fig. 6B) The dynamic range narrowed to 0.12-24.63 ppb ($\Delta R = 2.071\text{-}1.453 \lg[C_{CLH}]$, R^2=0.998) with a detection limit of 0.12 ppb (n=3). The results demonstrate the label-free lateral flow impedimetric strip can work well in real life and show great potential in food safety supervision.

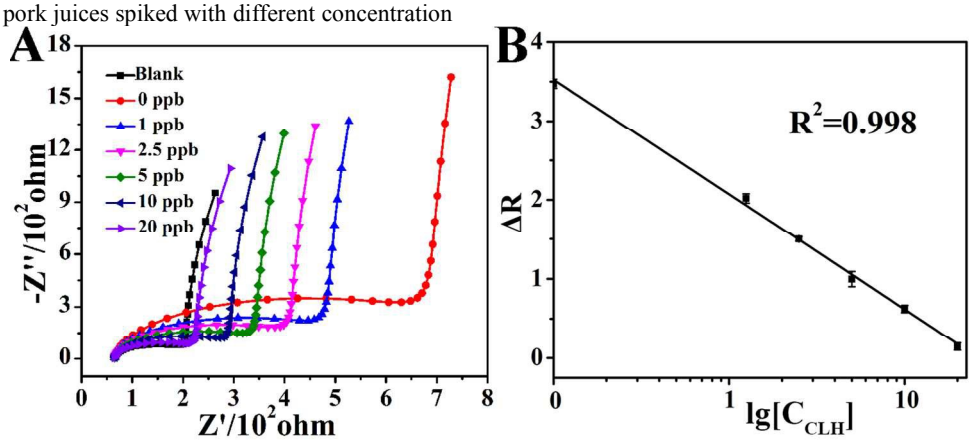

Fig.6 Performance of the one-piece lateral flow impedimetric strip for detection of CLH spiked in pork juice: (A) The EIS curves of pork juice containing different concentration of CLH assayed by fully assembled impedimetric strip; (B) calibration curve of CLH concentration *vs* normalized EIS signal change (ΔR), n=3.

4. Conclusions

In this study, a label-free lateral flow impedimetric immune-strip based on a PANI@GO composite film was developed for the first time to ultra-sensitively detect CLH with a detection limit of 0.12 ppb and a dynamic range of 0.12-58 ppb. The excellent analytical performance is accompanied by the synergistic effect of the components in the multifunctional nanocomposite, in which PANI provides electroactivity, due to its reversible electrochemical doping/dedoping property and also supplies anchor sites for covalent immobilization of proteins, and GO provides good electric conductivity as well as rigidity for enhancing stability. This one-piece lateral flow impedimetric testing strip can be also used to fabricate rapid,

sensitive and inexpensive label-free immunosensors for broad portable clinic diagnosis and food contamination analysis, particularly in point-of-care and daily food quality inspection.

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Notes and references

[a] Institute for Clean energy & Advanced Materials, Faculty of Materials & Energy, Southwest University, Chongqing 400715, China
[b] Chongqing Key Laboratory for Advanced Materials and Technologies of Clean Energies, Chongqing 400715, China
[c] Chongqing Engineering Research Center for Rapid diagnosis of Fatal Diseases, Chongqing 400715, China
*Corresponding authors: Tel: +86-23-68254842; Fax: +86-23-68254969; E-mail: lingyu12@swu.edu.cn

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