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Ultrasensitive detection of the β-adrenergic agonist brombuterol by a SERS-based lateral flow immunochromatographic assay using flower-like gold-silver core-shell nanoparticles

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Abstract The β-adrenergic agonist brombuterol (BB) is illicitly used as an additive in animal feed to enhance the lean meat-to-fat ratio. The authors describe an ultrasensitive lateral flow immunochromatographic assay (LFIA) based on the use of surface enhanced Raman scattering (SERS) for the determination of brombuterol in swine meat and urine samples. Flower-like gold-silver core-shell bimetallic nanoparticles (referred to as AuNF@Ag) displaying strong SERS enhancement were synthesized, characterized and used as the substrate for the preparation of the LFIA. Polyclonal antibody against brombuterol was immobilized on the surface of the AuNF@Ag particles carrying the Raman reporter 4-mercaptobenzoic acid (MBA). After performing an LFIA, the Raman scattering intensity of MBA on the test line was measured and used for quantitation of brombuterol. Figures of merit of this assay procedure include (a) duration of LFIA process of 15 min; (b) an IC_{50} value (e.g. the concentration of brombuterol producing 50% of signal inhibition in standard curve) of 380 pg mL-1; and (c) a limit of detection as low as 0.5 pg mL-1. The LFIA is selective over the molecules salbutamol, ractopamine, phenylethanolamine A, isoproterenol and phenylephrine, but shows a 8.5% cross-reactivity to clenbuterol, probably due to the high structural similarity. Swine meat and urine samples spiked with different amounts of brombuterol were analyzed by this method and gave recoveries between 95.8 and 108.0%, and relative standard deviations between 2.0 and 6.3% (for $n = 3$).

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Introduction

β-Adrenergic agonists, a family of synthetic phenethanolamine compounds, are therapeutically administered to treat asthma and bronchial diseases in humans and animals [1]. Nevertheless, they are well-known as repartitioning agents illicitly employed in livestock feeds as growth promoters by virtue of their ability to increase the lean meat-to-fat ratio [2]. Owing to the potential hazard to human cardiovascular and central nervous systems posed by the presence of these β-agonist residues in edible animal tissue through the food chain [3], the legal tolerance for β-agonists exploited as growth-promoting agents in livestock production in the European Union and China is "zero" [4, 5]. A new kind of β-agonist called brombuterol has come to be used as a substitute for other well-known β-adrenergic agonists including clenbuterol, salbutamol and ractopamine (Fig. 1) as a means of evading detection by routine screening methods. A prohibition on the use of brombuterol as a growth-promoting agent in animal feed and drinking water was stipulated in the Bulletin 1519 issued by the Ministry of Agriculture of China in 2010 [5].
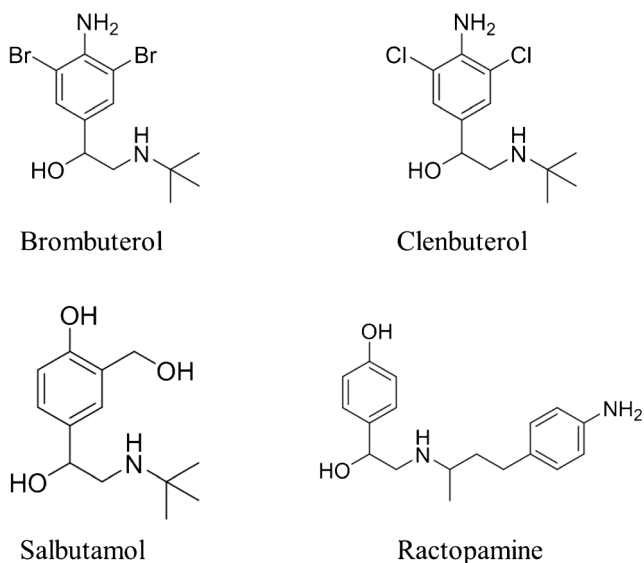
Fig. 1 Molecular structures of brombuterol, clenbuterol, ractopamine and salbutamol

The main analytical methods for the detection of brombuterol in food and biological samples involve chromatograpy, including liquid chromatography − tandem mass spectrometry (LC-MS/MS) [6, 7], liquid chromatography − electrospray ionization mass spectrometry (LC-ESI-MS) [8], gas chromatography − tandem mass spectrometry (GC-MS/MS) [9] and ultra-high performance liquid chromatography-high-resolution mass spectrometry (UHPLC-LTQ Orbitrap MS) [10]. Apart from chromatographic methods, enzyme-linked immunosorbent assays (ELISAs) [11, 12] and electrochemiluminescent immunosensors [13–15] have also been reported for determinations of brombuterol residues. However, though these chromatographic methods have excellent accuracy and low detection limits, they require sophisticated instrumentation, skilled analysts and time-consuming procedures, which hinder their applicability for field analysis and rapid screening. ELISA is highly selective and sensitive, but a primary drawback is the time-consuming and laborious incubation washing and rinsing procedures which are involved. The electrochemical methods are disturbed by many interferents. As a consequence, the development of simple, rapid, low-cost, sensitive and specific methods for the detection of β-agonists is an urgent priority.

Compared with the aforementioned detection methods, lateral flow immunochromatographic assay (LFIA) has the advantages of minimal user training and equipment, rapid execution, on-site detection, low cost and reasonable shelf life, making it a popular technology for point-of-care (POC) diagnostics [16, 17]. However, the normal Au colloidal particle-based LFIA is usually used for qualitative or semi-quantitative analysis. Numerorous efforts have been reported on converting the immunoprobe on the test line into digital

signals utilizing nanoparticles (NPs) for quantitative analysis. These nanoparticles include colored nanoparticles (e.g., AuNPs, carbon NPs) and fluorescent nanoparticles (e.g., quantum dots, up-converting phosphor NPs, dye-doped NPs, and liposome, etc) [18–20]. Although the LFIA based on fluorescent nanoparticles has high sensitivity, the drawbacks are fluorescence background interference, toxicity, price and difficulty of preparation, while the LFIA using colored nanoparticles displayed low sensitivity.

Surface enhanced Raman scattering (SERS) is a fascinating phenomenon where the Raman signal of the analyte absorbed on a rough metallic surface can be amplified tremendously even for single molecule detection [21, 22]. The SERS technique has gained a lot of attention on account of its high selectivity, ultra-sensitivity, non-destructivity, and reliable and fast detection in sensing chemical and biological molecules in trace amounts [23]. To date, a variety of applications employing SERS have been achieved in research fields such as environmental monitoring, diagnostics, biodetection and bioimaging [24, 25].

In the case of the SERS technique, Au and Ag nanoparticles are the most widely used optical enhancement substrates. It has been theoretically predicted and experimentally confirmed that sharp metallic protrusions and nano-gaps, called "hot spots", exhibit extremely high SERS enhancement in comparison to spherical particles due to the anisotropic distribution of the electromagnetic field near the surface of nonspherical particles. These nanomaterials exist in the form of rods, prisms, cubes, plates, stars, flowers, etc. [26, 27]. In order to combine the advantages of gold (uniformity, stability, biocompatibility, etc.) and silver (larger SERS enhancement), many researchers have produced a series of Au-Ag bimetallic nanoparticles such as Au@Ag NPs, Au^{dye}@Ag NPs and Au-Ag alloy NPs and applied them in SERS [28, 29]. The gold-silver core-shell bimetallic nanoparticles have been found to have a higher SERS activity, mainly due to the electronic ligand effect and localized electric field enhancement of core-shell nanoparticles [30]. For example, Zhang's group has reported that urchin-like gold nanoflowers (AuNFs) have good structural stability and monodispersity through a seed-mediated growth approach [31].

In this work, using flower-like gold-silver core-shell bimetallic nanoparticles (AuNF@Ag) as a higher enhanced SERS substrate, we developed a LFIA based on SERS for the detection of brombuterol in swine meat and urine samples. To develop this LFIA, an immunoprobe (e.g., AuNFs^{MBA}@Ag-Ab) was synthesized (Fig. 2a) by immobilizing the polyclonal antibody against brombuterol on the surface of AuNF@Ag core-shell bimetallic nanoparticles which have been sandwiched with a Raman reporter (MBA). After LFIA procedures, the specific Raman scattering intensity of MBA at 1074 cm^{-1} on the test line was measured for the quantitative detection of brombuterol.
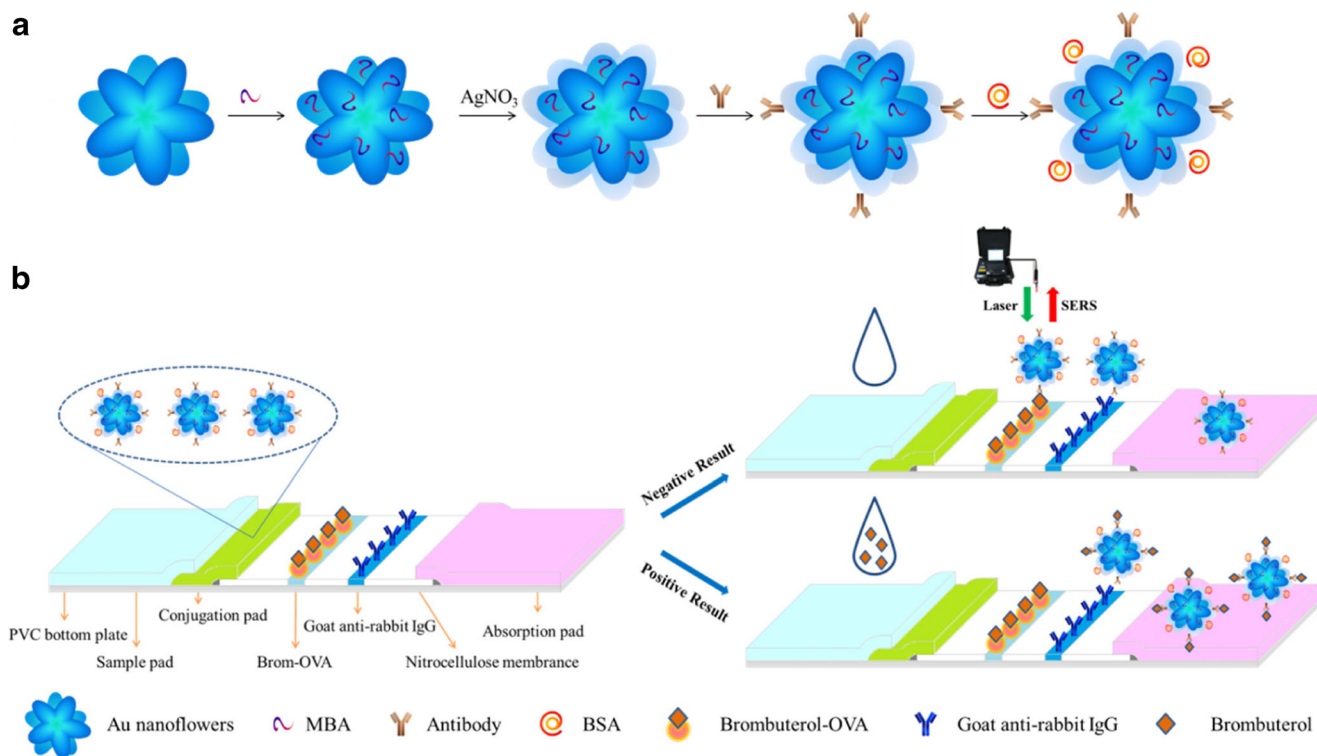
Fig. 2 **a** Schematic illustration of the preparation of AuNFs^{MBA}@Ag-Ab immunoprobe; **b** Assembly of LFIA strip and the principle of competitive SERS-LFIA for brombuterol

Experimental

Reagents, materials and apparatus

Chloroauric acid (HAuCl$_4$·4H$_2$O, 47.8%), trisodium citrate (99.0%), hydroquinone (HQ, 99.0%) and silver nitrate (99.8%) were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, www.sinoreagent.com). L-ascorbic acid (AA, 99.7%) was bought from Shanghai Ling Feng Chemical Reagent Co., Ltd. 4-Mercaptobenzoic acid (MBA, 99%) was obtained from Aladdin China Ltd. (Shanghai, China). Bovine serum albumin (BSA), chicken egg ovalbumin (OVA) and Tween-20 were bought from Sigma (St Louis, MO, USA, www.sigma-aldrich.com). Polyvinylpyrrolidone (PVP, 98%) was purchased from Sangon Biotech (Shanghai, China). Brombuterol, clenbuterol, ractopamine, salbutamol, isoproterenol and phenylephrine were purchased from National Institutes for Food and Drug Control. (Beijing, China). Phenylethanolamine A was bought from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). Goat anti-rabbit IgG was obtained from Zhong Shan-Golden Bridge Biological Technology Co. (Beijing, China). All other chemical reagents used in this work were of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Nitrocellulose (NC) membranes were purchased from Whatman (Shanghai, China). PVC sheets, adhesive tape and filter paper were purchased from Jieyi Biotechnology Co. Ltd. (Shanghai, China). The ultraviolet visible spectrophotometer (UV-2300) was purchased from Techcom Com. (Shanghai, China, www.techcomp.cn). The deionized-RO water machine (Dura 12FV) was purchased from THE LAB Com. (USA). The digital photographs were taken with an Apple iPhone 6 camera (Apple Inc., USA). Transmission electron microscopy (TEM) images were taken on a Tecnai G220 from FEI Company (USA). The portable Raman Analyser RamTracer-200-HS was obtained from OptoTrace Technologies, Inc. (Suzhou, China, www.optotrace.com.cn). The membrane strip reader (DT211) for gold nanoparticle based-LFIA was purchased from Beijing China Instrument Tech. Co., Ltd.

Preparation of the coating antigen and polyclonal antibody

The polyclonal antibody against brombuterol and coating antigen (brombuterol − OVA conjugate) were prepared by our group [11]. Briefly, brombuterol was directly coupled to carrier proteins (BSA and OVA) by amino group diazotization. Brombuterol-OVA conjugate was used as a coating antigen to develop LFIA, while brombuterol-BSA conjugate was used as an immunogen for the production of polyclonal antibody. Two

adult New Zealand rabbits were immunized with brombuterol-BSA. After the fifth immunization, the antisera from the immunized rabbits were collected and stored at −20 °C until use. The IgG fraction of the antisera with high specificity to brombuterol, obtained by purifying antisera with protein-G affinity column, was applied in LFIA.

Preparation of AuNFs^{MBA}@Ag-Ab immunoprobe

The preparation of the AuNFs^{MBA}@Ag-Ab immunoprobe is presented in the Electronic Supporting Material (ESM) and illustrated in Fig. 2a.

Fabrication of LFIA strip

The fabrication of the LFIA strip is presented in the ESM and illustrated in Fig. 2b.

Competitive LFIA for brombuterol

The principle of the competitive SERS-based LFIA for brombuterol is similar to that based on normal gold colloidal particles. As illustrated in Fig. 2b, when negative samples (no brombuterol in standard/sample solution) are added on the sample pad, the immunoprobe (AuNFs^{MBA}@Ag-Ab) flows along with the sample solution via capillary action and is captured by coating antigen (brombuterol-OVA) on the T line. Excess immunoprobe will continuously migrate to the C line and be captured by goat anti-rabbit IgG. In this case, the color will appear on both lines due to accumulation of immunoprobe. Conversely, when positive samples (e.g. the standard/sample solutions containing large amounts of brombuterol) are applied on the sample pad, the AuNFs^{MBA}@Ag-Ab will first bind to the analyte, preventing it from being captured by the coating antigen. Hence, there is no AuNFs^{MBA}@Ag-Ab bound on the T line, but the immunoprobe will still move to the C line and be bound by goat anti-rabbit IgG. Thus the degree of intensity of Au^{MBA}@Ag-Ab color as well as the SERS signal on the T line is the reverse of the concentration of brombuterol in the standard/sample solution. The SERS scattering intensity arising from the MBA captured on the test line will be measured by a portable Raman Analyser for quantitative analysis of brombuterol.

The procedures of the SERS-LFIA for brombuterol are as follows. 150 μL of standard/sample solution was pipetted onto the sample pad. The standard/sample solutions, together with the immunoprobe driven by capillary action, flowed toward the absorbent pad. The color on T line or C line gradually became visible to the naked eye. 15 min later, the SERS signal of MBA (1074 cm^{-1}) on the T line was acquired by a portable Raman spectrometer. The excitation source was tuned to 785 nm with a laser power of 20 mW at the test line

area. The typical integration time used in this assay was 10 s. The SERS peak intensity at 1074 cm^{-1} which arose from MBA was measured from an average of spectra collected at 10 different spots along the middle part of the T line for quantification.

Spiking experiment

The spiking experiment is presented in the ESM.

Result and discussion

### Synthesis and characterization of AuNFs nanoparticles

AuNFs are synthesized by a seed-mediated growth method in water at room temperature, in which citrate acts as the ligand, and both citrate and HQ are used as reductants. Sodium citrate, which manifests weak reducibility at room temperature, can only reduce Au(III) to Au(I), while HQ with a high selective reducibility is able to in reduce Au(I) to Au(0) on the gold seed [32]. A high concentration of HQ is required to promote rapid deposition of excess Au(0) on the highly active (111) planes via a kinetics-favored process for branched growth, and to stabilize the morphology of AuNFs. The color of the AuNFs solution is blue (Fig. 3a, insert). From the TEM image (Fig. 3a), it can be seen that the AuNFs has a flower-like shape (having a solid core and several tips) with an average diameter of 60 ± 8 nm ($n = 50$). The monodispersity and shape consistency of the AuNFs are also good.

The UV-vis spectrum of AuNFs is shown in Fig. 3c (blue line). It is seen that AuNFs exhibits a broad absorption band with a localized surface plasmon resonance (LSPR) centered at 626 nm. For comparison, the UV-vis spectrum of spherical AuNPs of a similar size was also measured (Fig. S1). It can be seen that the LSPR peak of spherical AuNPs lies at 541 nm. Obviously, there is a significant red-shift of the LSPR for AuNFs, which can be qualitatively explained by plasmon hybridization theory [33]. The AuNFs may be a better candidate for fabricating SERS-active tags due to potential "hot spots", a larger surface area and the closeness between the laser excitation and LSPR wavelength [17, 34].

Synthesis and characterization of AuNFs^{MBA}@Ag nanoparticles

In order to stabilize the morphology of particles during the preparation of AuNFs^{MBA}@Ag NPs, an amount of HQ was supplemented. Without supplemental HQ, the flower-like structures of AuNFs^{MBA} almost disappeared and the reduced Ag nanoparticles were not deposited on the surface of the AuNFs (Fig. S2). If excess MBA was not removed and HQ was not supplemented, after the reducing AgNO$_3$ with AA, the final particles displayed

Fig. 3 Characterization of AuNFs and AuNFs^{MBA}@Ag NPs. **a** TEM image of AuNFs, with inset of a digital photograph of AuNFs solution; **b** TEM image of AuNFs^{MBA}@Ag NPs; **c** The ultraviolet-visible (UV-vis) absorption spectra of AuNFs and AuNFs^{MBA}@Ag NPs
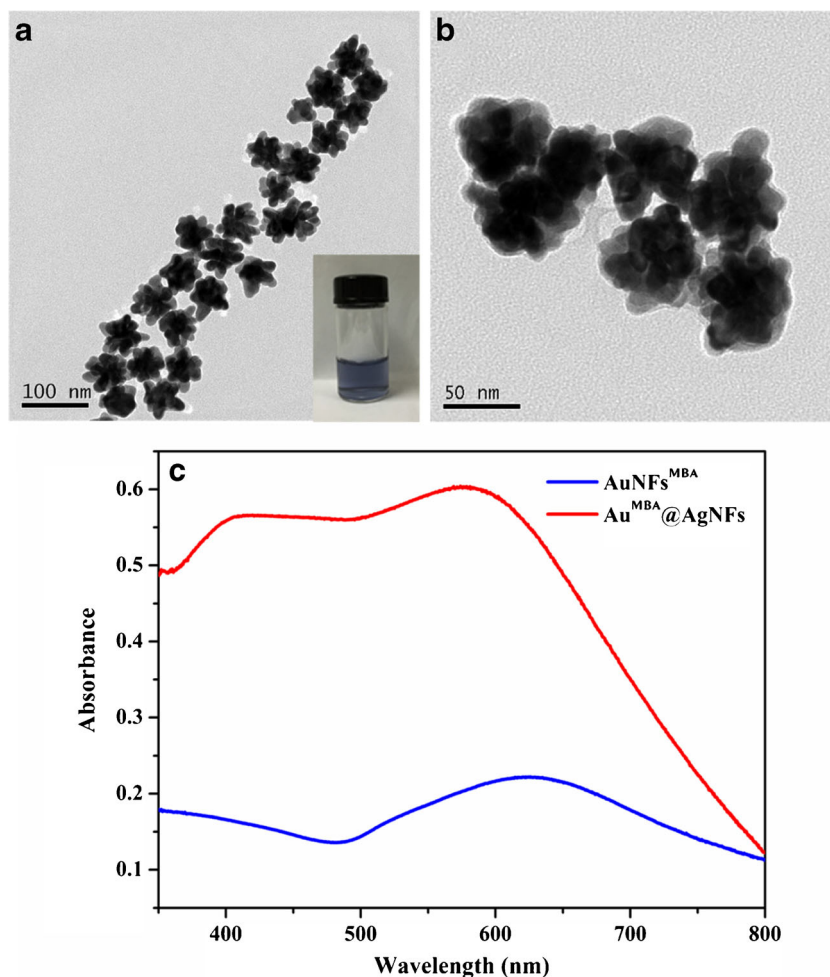
spherical profile instead of a flower-like shape (Fig. S3). The amount of MBA and AgNO$_3$ used was optimized. MBA can be easily attached to AuNFs via the thiol group in the MBA molecule. When 10.0 mL of the AuNFs was mixed with different amounts of MBA (5–35 nmol), more MBA addition to AuNFs solution caused the AuNFs to aggregate and even to precipitate. It was found that 25 nmol was the appropriate amount of MBA which needed to be mixed with 10.0 mL of the AuNFs in order to form AuNFs^{MBA} NPs. For 2 mL of AuNFs^{MBA}, 50 μL (10 mmol L^{-1}) of AgNO$_3$ was the optimal volume (Fig. S4).

The TEM image of AuNFs^{MBA}@Ag is shown in Fig. 3b. It can be seen that the AuNFs^{MBA}@Ag retains its flower-like shape and the silver is uniformly coated onto the AuNFs^{MBA}. The average diameter of AuNFs^{MBA}@Ag is 64 ± 8 nm, indicating the 2 nm thickness of the Ag shell. From the UV-vis spectrum (red line in Fig. 3c), it can be seen that the LSPR of AuNFs^{MBA}@Ag is located at 575 nm instead of 626 nm. The blue-shift of LSPR provides strong evidence for the Ag shell growth on AuNFs^{MBA}, which has been investigated theoretically according to the traditional Mie scattering theory and with the aid of dielectric data [35, 36]. In addition, the surface plasmon band of silver at 400 nm was

also observed. These results clearly demonstrate the successful coating of the silver shell onto the AuNFs.

For comparison, we also synthesized spherical Au and Ag nanoparticles attached with MBA (e.g. AuNPs^{MBA} and AgNPs^{MBA}). The SERS signals from the colloidal solutions containing AuNFs^{MBA}@Ag, AuNFs^{MBA}, AuNPs^{MBA} and AgNPs^{MBA} were measured using the portable Raman Analyser. As illustrated in Fig. 4, the SERS intensity from AuNFs^{MBA}@Ag is higher than that of AuNFs^{MBA}, AuNPs^{MBA} and AgNPs^{MBA}, demonstrating the highest SERS enhancement to be that possessed by the AuNF@Ag core-shell bimetallic nanoparticles.

Inspection of specific and nonspecific binding

Before bromchuterol detection was attempted, the specific and nonspecific binding of the immunoprobe was inspected. The LFIA procedures at zero concentration of analyte were performed under four conditions: (a) AuNFs^{MBA}@Ag-Ab as a probe, bromchuterol-OVA coated on the T line; (b) AuNFs^{MBA}@Ag-BSA (BSA substituting Ab) as a probe, bromchuterol-OVA coated on the T line; (c) AuNFs^{MBA}@Ag-

Ab as a probe, OVA spread on the T line; (d) AuNFs^{MBA}@Ag-Ab as a probe, Na$_2$CO$_3$-NaHCO$_3$ buffer solution dispersed on the T line. The Raman spectra in the above four situations are illustrated in Fig. S5. From Raman spectrum (a) in Fig. S5, it can be seen that both specific Raman characteristic peaks of MBA at 1074 cm^{-1} and 1583 cm^{-1} with the signal values of 15,139 a.u. and 8566 a.u. appeared, clearly demonstrating that the immunoprobe (AuNFs^{MBA}@Ag-Ab) is specifically bound with brombuterol-OVA at the T line on the membrane. In contrast, from the Raman spectra (b-d) in Fig. S5, almost no SERS signals appeared either at 1074 cm^{-1} or at 1583 cm^{-1}, indicating that there is almost no nonspecific adsorption.

Optimization of method

The following parameters were optimized: (a) amount of the Ab used for immunoprobe preparation; (b) amount of coating antigen; (c) immunoprobe utilized in the conjugate pad; (d) duration of LFIA process. In this study, the inhibition ratio (B$_0$/B$_{0.1}$) was used to evaluate the optimization, where B$_0$ and B$_{0.1}$ refers to the SERS intensities of MBA at 1074 cm^{-1} when brombuterol concentrations are at 0 ng mL^{-1} and 0.1 ng mL^{-1}, respectively. Respective data and figures are given in the ESM. The following experimental conditions were found to give the best results: (a) to 1.0 mL AuNFs^{MBA}@Ag NPs, 1 μL of Ab (3.8 mg ml^{-1}) was added (Fig. S6a); (b) 10 μL of brombuterol-OVA at 0.75 mg mL^{-1} was applied to the strip (Fig. S6b); (c) 0.8 μL of AuNF^{MBA}@Ag-Ab was used in the conjugate pad (Fig. S6c); (d) LFIA process duration of 15 min (Fig. S6d).

SERS-based LFIA for brombuterol

Under optimized conditions, the brombuterol standard solutions ranging from 0 to 100 ng mL^{-1} in 1% PVP-PBST [i.e. a mixture of 1% of PVP with 0.01 mol·L^{-1} phosphate-buffered

saline (pH 7.4, containing 145 mmol·L^{-1} NaCl and 0.1% (v/v) of Tween-20), v/v = 1:1] were applied to SERS-based LFIA. After the addition of 150 μL of brombuterol solution to the sample pad, 15 min later, the color intensity visible to the naked eye at T-line varied inversely with the concentration of brombuterol (Fig. 5a). The corresponding Raman spectra of the T lines was measured by SERS analyzer and presented

Fig. 5 a Photograph of the strips following the LFIA procedure at different brombuterol concentrations (ng mL^{-1}); b The Raman spectra of the T line in relation to the above strips; c Standard curve of the SERS-LFIA for brombuterol. The bars represent the standard deviation of the 10 measurements of the intensities of MBA at 1074 cm^{-1} on test lines
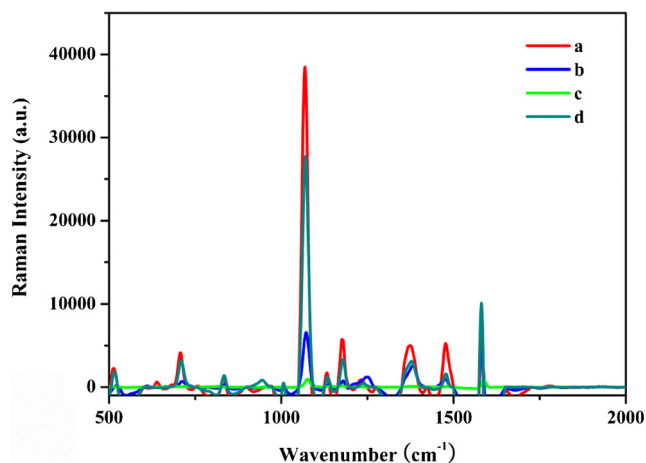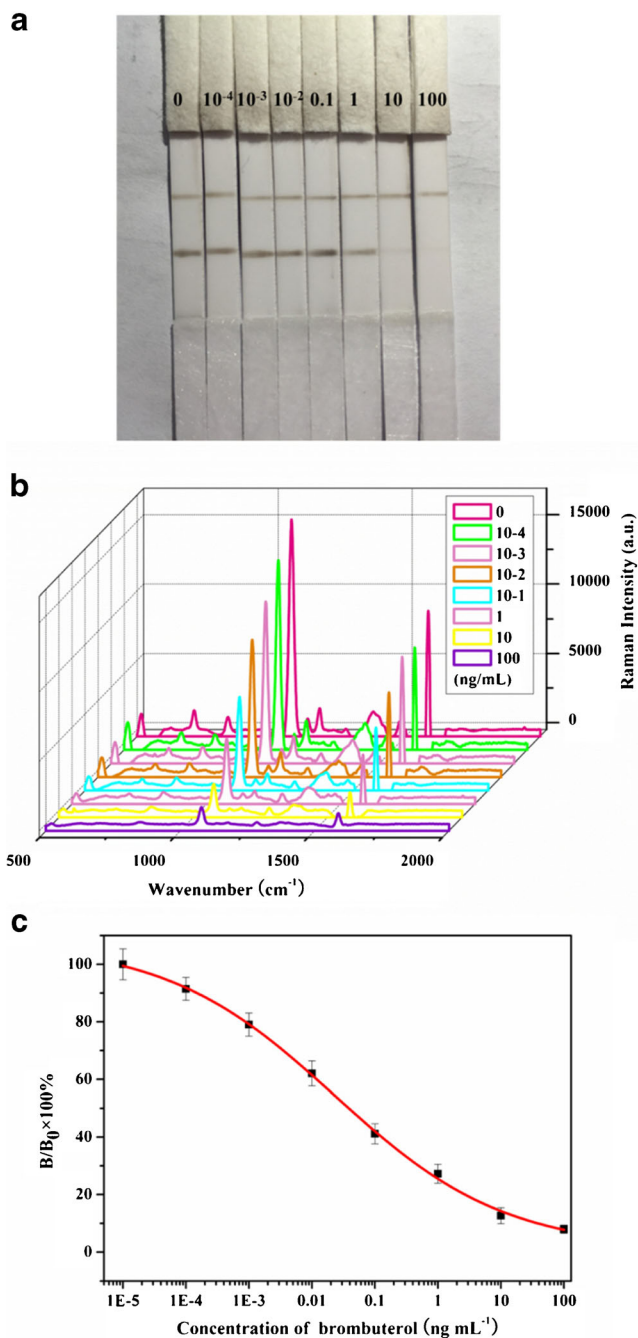

Fig. 4 The Raman spectra of AuNFs^{MBA}@Ag colloidal solution a, AuNFs^{MBA} colloidal solution b, the spherical AuNPs^{MBA} colloidal solution c and AgNPs^{MBA} colloidal solution d

in Fig. 5b, which clearly shows that the SERS intensity at 1074 cm^{-1} generated from MBA gradually declined with increasing brombuterol concentration. The standard curve of the SERS-LFIA for brombuterol is illustrated in Fig. 5c, which is plotted in the form $B/B_0 \times 100\%$ versus log C (B and B_0 are the Raman signals of MBA at 1074 cm^{-1} when brombuterol is at the standard point and at zero concentration). From Fig. 5c, it can be seen that the IC_{50} and LOD at $3 \times$ SD are 0.038 ng mL^{-1} and 0.15 pg mL^{-1}, respectively.

A comparison of the IC_{50} and LOD obtained from this assay with those obtained from ELISA [11, 12] and immunosensors [13–15] and based on the same polyclonal antibody and coating antigen for the detection of brombuterol, is given in Table 1. It is seen that the IC_{50} and LOD values achieved in this study are lower than those obtained by other immunoassays, indicating the ultrasensitivity of the SERS-based LFIA.

Although traditional LFIA based on labels consisting of common gold nanospheres with a diameter of about 20 nm is normally used for qualitative or semi-quantitative analysis, the membrane strip reader (DT211) which is commercially available can also be used for quantitative analysis. A supplemental experiment was performed in which gold nanoparticles with a diameter of 20 nm were used for the development of an Au NP-based LFIA and the corresponding standard curve of the LFIA for brombuterol was constructed (Fig. S7). It can be seen that the IC_{50} and LOD values are 0.63 ng mL^{-1} and 12 pg mL^{-1}, which are about 1–2 orders of magnitude higher than those achieved by SERS-based LFIA.

Reproducibility of the SERS signals

The reproducibility of the SERS signals was investigated. As shown in Fig. S8a, when three standard solutions at 0, 0.1, and 1.0 ng mL^{-1} are subjected to LFIA procedures, the relative standard deviation (RSD) of the SERS intensities at 1074 cm^{-1} collected from ten different points on the middle parts of the T line are 5.07%, 5.80% and 6.26%, respectively, showing the high precision of the SERS signal.

Stability of the assay

The stability of the assay was examined by running the LFIA using the same batch of strips which were stored for 2, 4, 6, 8 and 10 weeks under two different conditions (1) storage in sealed packages with dessicant under room temperature; (2) storage in sealed glass bottles with nitrogen gas at 4 °C in a refrigerator. It was found that the Raman intensities on the test line declined gradually, over 6 weeks under the first set of conditions and over 10 weeks under the second set of conditions, indicating the higher stability of the assay when the strips were stored under the second set of conditions.

Specificity of the assay

The specificity of a competitive immunoassay is often expressed by cross-reactivity (CR) value. Six structurally related compounds (clenbuterol, salbutamol, ractopamine, phenylethanolamine A, isoproterenol and phenylephrine) were used to evaluate the specificity of the SERS-LFIA for brombuterol. All compounds including brombuterol were prepared in a concentration range of 1–10,000 ng mL^{-1} and subjected to SERS-LFIA procedures. The CR values were calculated using the formula CR (%) = [IC_{50} of brombuterol]]/[IC_{50} of tested compound] $\times 100\%$. As illustrated in Fig. S8b, there is no CR of the LFIA with salbutamol, ractopamine, phenylethanolamine A, isoproterenol and phenylephrine, but 8.48% CR occurs in the case of clenbuterol. Considering the high similarity in molecular structure between brombuterol and clenbuterol (Fig. 1), a CR value of 8.48% for the assay with clenbuterol is still relatively low, demonstrating the high specificity of this LFIA for brombuterol determination.

Detection of brombuterol in spiked samples

In order to examine the applicability of the SERS-LFIA for sample analysis, swine meat and urine samples were spiked with different amounts of brombuterol. After the pretreatment of the samples, the extraction solutions (or diluted solutions) of the spiked samples were detected by LFIA. The

Table 1 An overview on recently reported immunoassays for determination of brombuterol

Material/method	IC_{50} (ng mL^{-1})	LOD (pg mL^{-1})	Reference
Microtiter plate, polyclonal antibody/ELISA	0.165	7	[11]
Microtiter plate, monoclonal antibody/ELISA	0.56	47	[12]
Quantum dots-graphene@gold nanoparticles/immunosensor	-	3	[13]
Gold nanoparticles, silver-cysteine hybrid nanoribbon/immunosensor		1.5	[14]
Gold nanoparticles-functionalized zinc oxide nanorod/immunosensor		0.3	[15]
AuNF@Ag/SERS-LFIA	0.038	0.15	This work

Table 2   SERS-LFIA for the detection of brombuterol in spiked swine meats and urine samples and confirmed by LC-MS/MS

Sample [a]	Brombuterol spiked in tissue samples (ng g^{-1}) or urine samples (ng mL^{-1})	Theoretical brombuterol concentration in extracts or diluted urine (ng mL^{-1})	SERS-LFIA		LC-MS/MS	
			Recovery (%) (mean ± SD, $n = 3$)	RSD (%)	Recovery (%) (mean ± SD, $n = 3$)	RSD (%)
Swine meat	0.003	0.01	102.1 ± 5.3	5.2	98.5 ± 3.6	3.7
	0.03	0.1	100.1 ± 4.9	4.9	97.4 ± 3.1	3.3
	0.3	1.0	98.6 ± 1.9	2.0	102.1 ± 4.7	4.6
Swine urine	2	0.01	99.4 ± 2.5	2.5	105.7 ± 5.3	5.0
	20	0.1	95.8 ± 3.5	3.7	96.8 ± 4.5	4.7
	200	1.0	108.0 ± 6.8	6.3	103.3 ± 5.4	5.2

[a] No detectable of brombuterol was found in all samples without spiking, which were confirmed by LC-MS/MS [37]

brombuterol content of spiked samples was quantified according to the standard curve run on the same day. In addition, all spiked meat and urine samples were also measured by LC-MS/MS, which was performed according to the standard protocols issued by the Chinese government [37]. The results obtained for brombuterol are summarized in Table 2. The recoveries of brombuterol from spiked samples measured by SERS-LFIA are 95.8–108.0% with a RSD value in the range of 2.0% to 6.3% ($n = 3$). The recoveries and RSD values obtained by SERS-LFIA are similar to those obtained by MC-MS/MS. These results indicate that this new approach is a method which can be effectively applied in the detection of target analyte in swine meat and urine samples.

Conclusion

The competitive SERS-based LFIA for the detection of β-agonist brombuterol in swine meat and urine samples is described. The AuNF@Ag core-shell bimetallic nanoparticles were synthesized, characterized and applied as the substrate for the preparation of immunoprobe due to their high SERS enhancement. Under optimal conditions, the IC$_{50}$ and LOD values of this approach for brombuterol are 0.038 ng mL^{-1} and 0.15 pg mL^{-1}, respectively, indicating ultra-sensitivity. This LFIA also displays high specificity and reproducibility. The results from the spiking experiment demonstrate the high precision and accuracy of the assay. Conceivably, the assay can be used, in a sandwich format, for the detection of macromolecules, and by immobilizing more than two different kinds of coating antigens on the NC membrane as test lines and utilizing corresponding AuNFs^{MBA}@Ag-Ab immunoprobes, it might be used for multiplex assays. The proposed assay proves to be a rapid, simple and ultrasensitive analytical method for brombuterol detection in swine meat and urine samples.

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