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Colorimetric detection of ractopamine and salbutamol using gold nanoparticles functionalized with melamine as a probe

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ABSTRACT

A highly selective and sensitive method is developed for colorimetric detection of ractopamine and salbutamol using gold nanoparticles (AuNPs) functionalized with melamine (MA), respectively. The presence of these β-agonists induces the aggregation of gold nanoparticles through hydrogen-bonding interaction that was accompanied by a distinct change in color and optical properties, which could be monitored by a UV–vis spectrophotometer or even naked eyes. This process caused a significant decrease in the absorbance ratio ($A_{670\ nm}/A_{520\ nm}$) of melamine–gold nanoparticles (MA–AuNPs), and the color changed from wine red to blue. The systems exhibited a wide liner range, from 1×10^{-10} M to 5×10^{-7} mol/L with a correlation coefficient of 0.995 for ractopamine, and 1×10^{-10} M to 1×10^{-5} mol/L with a correlation coefficient of 0.996 for salbutamol, with measuring the absorbance ratio ($A_{670\ nm}/A_{520\ nm}$). The detection limit of these β-agonists is as low as 1×10^{-11} mol/L. Particularly, the developed method has been applied to the analysis of real swine feed samples and has achieved satisfactory results.
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1. Introduction

Recently, β-agonists received extensive attention for their illegally used as feed additives. They were original drugs for the treatment of pulmonary disease and asthma [1]. However, due to their potential roles in decreasing adipose tissue deposition and increasing protein accretion in livestock, β-agonists in particular clenbuterol, salbutamol and salbutamol were applied in animals to the production of muscle tissues [2]. Because their stable properties, the β-agonists can be easily deposited in human beings after meat consumption, and result in many serious health problems such as cardiovascular and central nervous diseases [3]. For their poisoning side-effects with people, many countries have forbidden the use of β-agonists in stockbreeding [4]. Therefore it is necessary to develop selective methods for detecting them at low concentrations with great precision and accuracy. Various analytical techniques for the determination of β-agonists have been reported, including liquid chromatography spectroscopy (LC) [5], gas chromatography spectrometry (GC) [6], electrochemical detection [7–9], and enzyme-linked immunosorbent assay (ELISA) [10]. Gupta and co-workers developed electrochemical detection for using electroactive phase in PVC based membranes, and had many achievements [11–15]. However, these methods are not very convenient for analysis of a large number of environmental samples as they generally require complex procedures and costly instruments, making it difficult for on-site and real-time determination.

Colorimetric sensors have attracted increasing considerations for their convenience of visual observation and simple operations in recent years [16–18]. Taking the advantage of their strong localized surface plasmon resonance (LSPR) absorption with extremely high extinction coefficients ($\sim3\times10^{-11}$ mol^{-1} L cm^{-1}) [19], systems based on the analyte-induced aggregation of gold nanoparticles (AuNPs) have been employed for the optical-detection applications. As the dispersed AuNPs are wine red whilst the aggregated ones are blue, this distinct change in color provide the naked-eye detection of various targets, including viruses [20], DNA [21], proteins [22], small molecules [23], metal ions [24] and cancerous cells [25]. However, the challenge to produce a colorimetric sensor is to find a suitable mediator to modify the surface of gold nanoparticles, which makes inter-particle crosslinking of AuNPs. More recently, our group has proposed a colorimetric method for the detection of clenbuterol using label-free gold nanoparticles in the presence of melamine (MA) [26]. Melamine can be connected with clenbuterol through hydrogen-bonding

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interaction [27,28] and has strong binding ability to the surface of AuNPs [29], which act as crosslinking agent between clenbuterol and AuNPs. In this paper, we improved our previous method and developed a new probe for the detection of two different β-agonists, ractopamine and salbutamol.

2. Experimental

2.1. Chemicals and materials

All reagents were of analytical grade and used without further purification. Solutions were prepared using high pure water with a resistance of 18 MΩ cm. Ractopamine, salbutamol and melamine was purchased from Sigma (USA). $HAuCl_4 \cdot 4H_2O$ was bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycine, L-glutamic acid, L-tyrosine, L-arginine, vitamin C and glucose were purchased from North Ringer Biotechnology Co., Ltd. (Beijing, China). $CaCl_2$, $MgCl_2$, and NaCl were purchased from Beijing Chemical Company (Beijing, China). The buffer was 0.01 M sodium acetate (pH 3.0–11.0, adjusted with acetic acid or sodium hydroxide). UV–vis absorption spectra were acquired on a UV-2550 spectrophotometer (Shimadzu, Japan), using 1-cm path length quartz cuvettes for measurements. IR spectra were measured with Avatar 360 FT-IR ESP (Thermo Nicolet, USA). Transmission electron microscopy (TEM) measurements were made on an H-7500 (Hitachi, Japan) at 80 kV. The pH of the solution was measured with PB-10pH meter (Sartorius, Germany).

2.2. Probe preparation

AuNPs were prepared by the reduction of $HAuCl_4$ with sodium citrate according to the method in the literature [30]. Typically, 15 mL of trisodium citrate (38.8×10^{-3} mol/L) was added rapidly into a boiling solution of 150 mL of $HAuCl_4$ (1×10^{-3} mol/L) under vigorously magnetic stirring and the mixed solution was continually boiled under stirring for another 30 min, producing a wine-red colored solution. The solution was cooled to room temperature while being stirred continuously and the citrate-stabilized suspension of gold nanoparticles were formed. The size of AuNPs was about 13 nm and the concentration was 10 nmol/L. The stock solution of AuNPs was stored in a refrigerator at 4 °C for further use.

To obtain melamine–gold nanoparticles (MA–AuNPs), 100 mL buffer and 50 mL of the prepared AuNPs were mixed to control the acidity of the aqueous solutions, then MA was added to this solution under stirring for about 15 min to make sure self-assembly of the MA onto the surface of gold nanoparticles. After this simple experiment, AuNPs functionalized with MA were prepared at room temperature.

2.3. Real swine feed samples preparation

Real swine feed samples were prepared according to previous literature [7]. Firstly, 1.0 g swine feed powder were weighed into 25.0 mL centrifuge tubes, and 3.0 mL acetone and 1.0 mol/L NaOH mixture (9:1, v-v) were spiked into the feed samples, than vibrating by supersonic for 5 min. Secondly, the mixture was centrifuged at 10,000 rpm and 5 °C for 10 min, repeated for three times and collected the clear supernatants. After that the supernatants were evaporated by nitrogen-blowing. Lastly, residues were dissolved in 1.0 mL acetate buffer (pH 5.0), then the same volume hexane were added for removing fat. After centrifuging at 10,000 rpm and 5 °C for 5 min, the remaining water phase were collected for analysis.

For the analysis of real swine feed samples, the above extracting solution was diluted 20 times with pH 5.0 acetate buffer and used to prepare a series of samples by "spiking" them with standard solutions of β-agonists.

3. Results and discussion

3.1. Mechanism

In this study, unlike the preceding method, melamine was priori modified onto the surface of Au nanoparticles by $-NH_2$ to prepare MA–AuNPs probe. The β-agonists had strong affinity to melamine through hydrogen-bonding interaction, which induced the inter-particle crosslinking of AuNPs, resulting in appreciable changes in color by naked eyes and absorption properties. Fig. 1 shows the mechanism of this platform for sensing β-agonists. Comparing with the previous research, these labeled AuNPs exhibit a more stable and convenient process. What is more, the MA–AuNPs probe can be applicable to measure other β-agonists, and the detection limit of ractopamine or salbutamol was managed down to 1×10^{-11} mol/L. In addition, to demonstrate the practicality of the present approach, it has been successfully applied to determine the concentration of β-agonists in real swine feed samples.

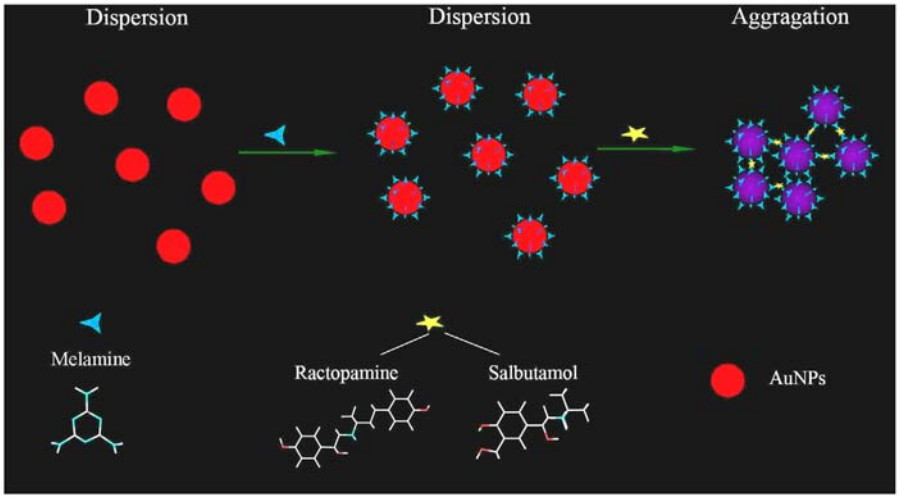

Fig. 1. Schematic illustration of the mechanism of the aggregation of MA–AuNPs induced by β-agonists.

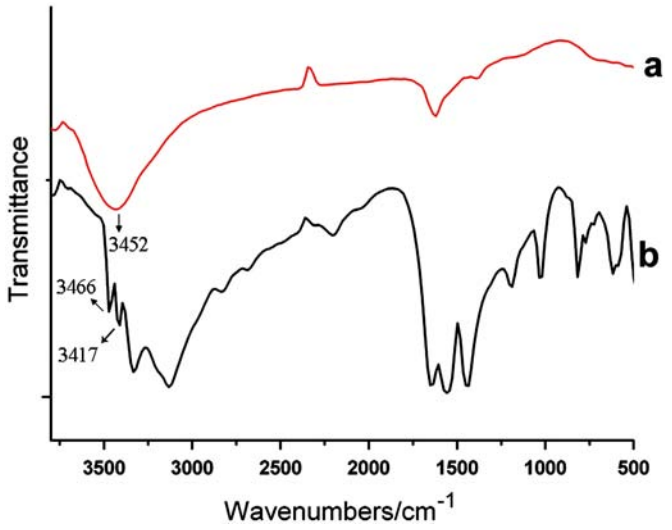

Fig. 2. FT-IR spectra of (a) MA–AuNPs and (b) MA.

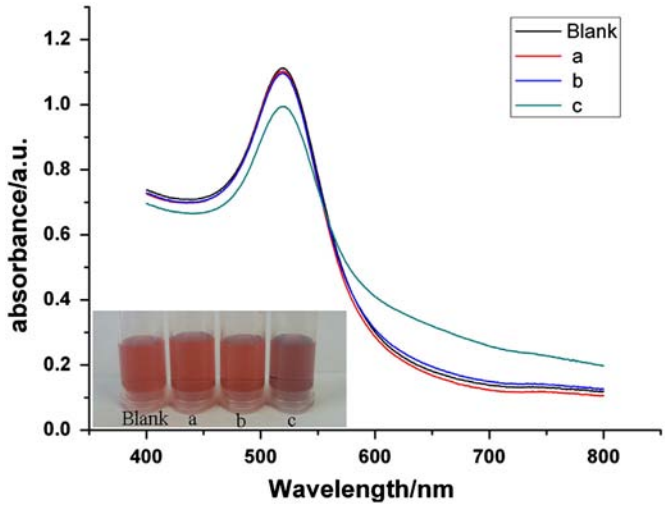

Fig. 3. UV–Vis spectra and visual color change of AuNPs in the presence of different concentrations of melamine (blank: 0; a: 1×10^{-7}; b: 3×10^{-7} and c: 5×10^{-7} mol/L).

3.2. Characterization

To investigate the modification of MA–AuNPs, FT-IR spectroscopy was performed. From Fig. 2, the characteristic absorption peak of –NH at 3000–3500 cm^{-1} in pure MA were shifted in the FT-IR spectra of MA–AuNPs, revealing that MA interacted with AuNPs through $-NH_2$ group, which indicating that MA has been successfully modified onto the surface of silver nanoparticles via the $-NH_2$ group of MA. Meanwhile, the FT-IR results further prove that MA acted as an excellent dressing agent for forming amino group in the surface of AuNPs.

3.3. The concentration of melamine

As melamine has a strong electrostatic interaction with citrate-capped AuNPs, which decreases the stability of AuNPs and causes visible color changes of AuNPs. So it is important to control the concentration of melamine used for the modification. AuNPs should be modified by an appropriate concentration of melamine to a critical state, at which the presence of melamine was more than enough and could not induce AuNPs to aggregate. Fig. 3 shows the UV–vis

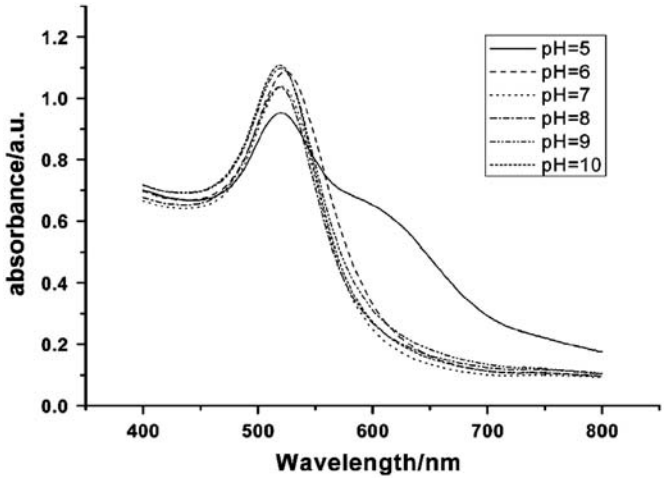

Fig. 4. UV–Vis spectra of MA–AuNPs solutions in the presence of 8×10^{-7} mol/L ractopamine with different pH ranging from 5.0 to 10.0.

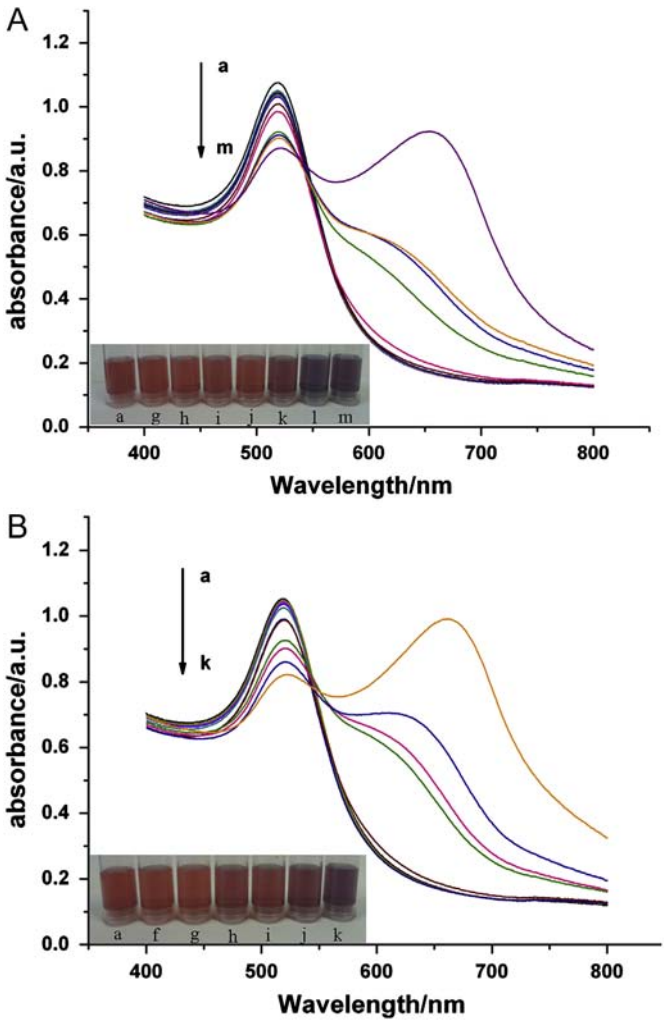

Fig. 5. UV–Vis spectra and photographs of MA–AuNPs solutions with various concentrations of ractopamine (A) (a: 0; b: 1×10^{-11}; c: 1×10^{-10}; d: 1×10^{-9}; e: 1×10^{-8}; f: 5×10^{-8}; g: 1×10^{-7}; h: 5×10^{-7}; i: 6×10^{-7}; j: 7×10^{-7}; k: 8×10^{-7}; l: 9×10^{-7} and m: 1×10^{-6} mol/L.) or salbutamol (B) (a: 0; b: 1×10^{-11}; c: 1×10^{-10}; d: 1×10^{-9}; e: 1×10^{-8}; f: 1×10^{-7}; g: 1×10^{-6}; h: 2.5×10^{-6}; i: 5×10^{-6}; j: 7.5×10^{-6} and k: 1×10^{-5} mol/L.).

spectrum and color changes of AuNPs solution modified by different concentrations of melamine. When the melamine concentration increased over 3×10^{-7} mol/L, the UV–vis spectrum of AuNPs

changed a lot and the color changed to purple. Based on these results, 3×10^{-7} mol/L melamine was chosen for the following experiments.

3.4. Effect of pH

The pH condition for colorimetric detection of β-agonists was optimized over the range from 5.0 to 10.0. When the pH value was less than 5.0, MA–AuNPs were unstable and aggregated easily, which could be attributed to the formation of intermolecular hydrogen bonding of MA in acidic solution. As shown in Fig. 4, when 8×10^{-7} mol/L ractopamine solution was added, Au nanoparticles show obvious UV–vis spectroscopy absorption change at pH 5, as ractopamine induces the aggregation of Au nanoparticles.

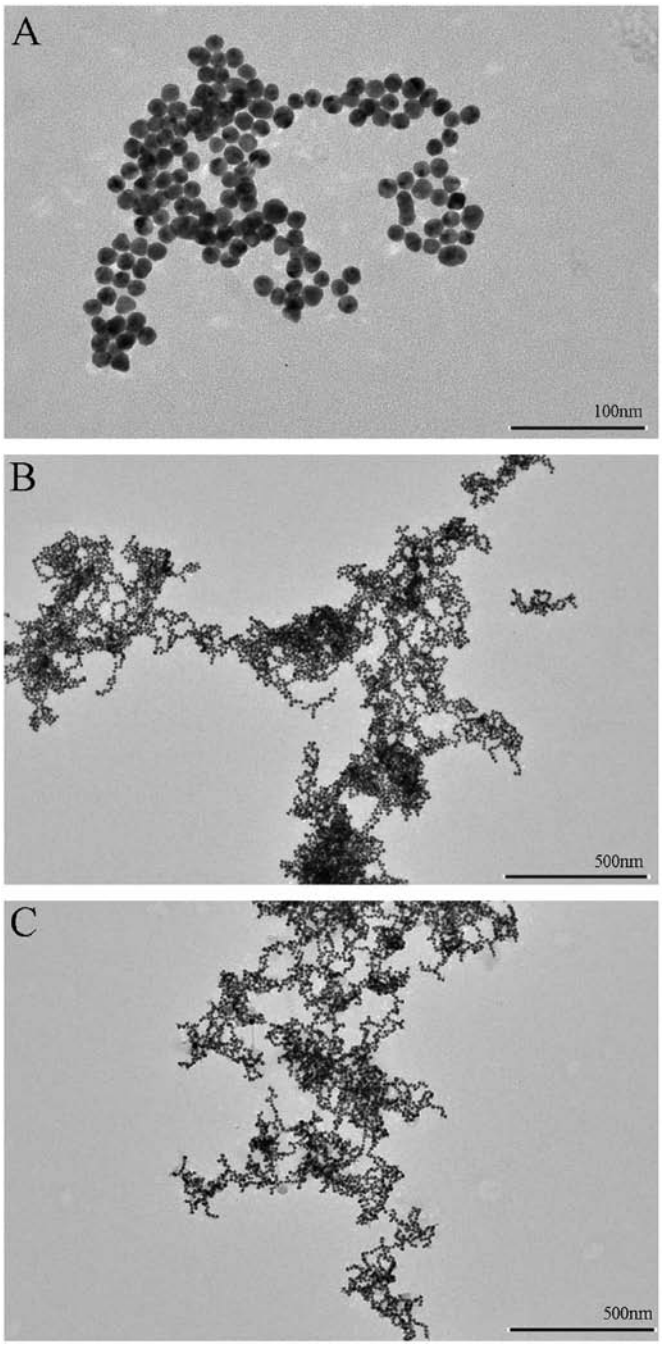

Fig. 6. TEM images of the MA–AuNPs formed in the absence of β-agonists (A) and presence of 1.0×10^{-6} mol/L ractopamine (B) or $1.0 - 10^{-5}$ mol/L salbutamol (C).

However, at the higher pH, the absorption showed a little change. Therefore, pH 5 was selected for further experiments considering the preferable sensitivity.

3.5. Colorimetric detection of β-agonists

To further demonstrate the assay for the direct colorimetric visualization of β-agonists, different amounts of ractopamine and salbutamol were added to aqueous suspensions of MA–AuNPs and tested after mixed for about 10 min, respectively. Fig. 5 reveals that the increase of β-agonists concentration would induce a decrease of AuNPs' maximal absorption at 520 nm, accompanying an obvious enhancement of the new peak at 670 nm, which indicated that the aggregating of AuNPs has a relationship with the concentration of β-agonists. Under the optimum conditions, the colorimetric assay was processed using MA–AuNPs to detect a series of ractopaminel with concentrations ranging from 1×10^{-11} to 1×10^{-6} mol/L (Fig. 5A) and salbutamoll with concentrations ranging from 1×10^{-11} to 1×10^{-5} mol/L (Fig. 5B). A clear color progression from red to purple was concomitant at the same time, thereby we can probably discriminate the concentration of β-agonists with the naked eye. A direct evidence for β-agonists-stimulated aggregation of the AuNPs could be further supported by transmission electron microscopy (TEM) measurements. Fig. 5 shows the TEM images of MA–AuNPs in the absence and presence of 1×10^{-6} mol/L ractopamine and 1×10^{-5} mol/L salbutamol. In the absence of β-agonists, AuNPs were well dispersed in aqueous solution (Fig. 6A). On the other hand, apparent

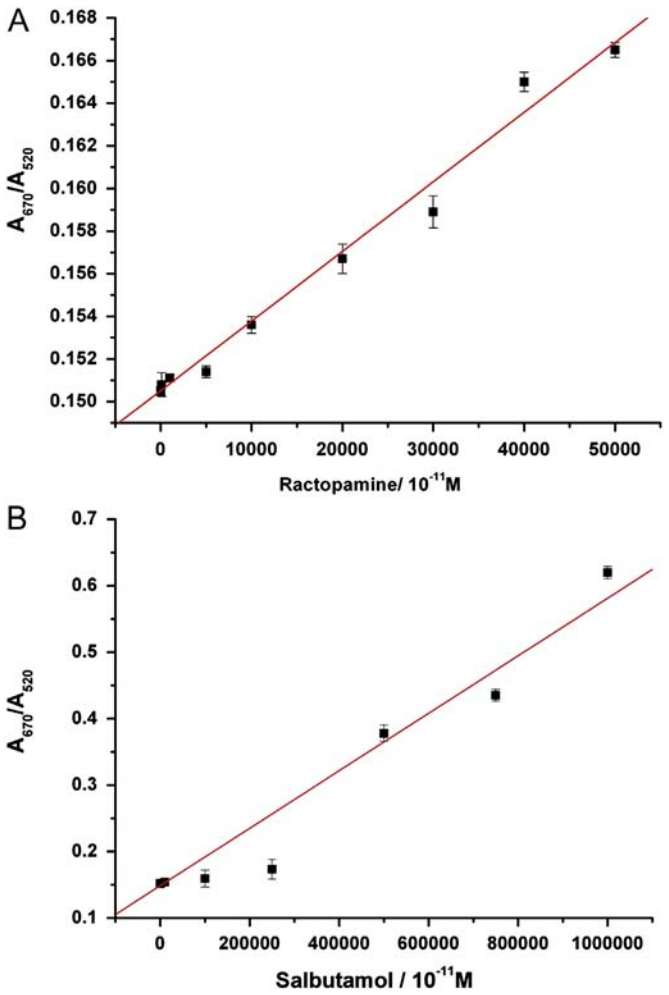

Fig. 7. Standard calibration curves of ractopamine (A) and salbutamol (B).

Table 1
Comparison of ractopamine and salbutamol obtained by different methods.

	Technique	Linear range (nmol/L)	Detection limit (nmol/L)	Reference
Ractopamine	High performance liquid chromatography	1.5–118.4	5.9	[31]
	Gas chromatography	29.6–1479.9	11.8	[32]
	Electrochemical method	148–5919.8	59.2	[33]
	Immunosensors	0.074–8.9	0.025	[34]
	Colorimetric detection	722–92,500	361	[35]
	Colorimetric detection	0.1–500	0.01	This work
Salbutamol	High performance liquid chromatography	104.5–1567	16.7	[36]
	Molecularly imprinted membrane	50–280	13.5	[37]
	Capillary electrophoresis	70,000–300,000	10,000	[38]
	Immunosensors	0.209–41.8	0.071	[34]
	Colorimetric detection	408–26,200	200	[35]
	Colorimetric detection	1–50,000	0.01	This work

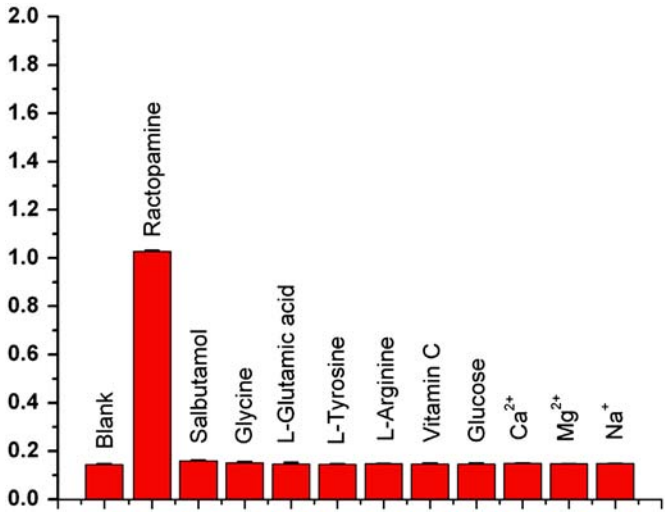

Fig. 8. Absorption ratio $A_{670\ nm}/A_{520\ nm}$ of AuNPs in the presence of β-agonists or other interferences (the concentrations of β-agonists and other interferences were 1×10^{-6} mol/L).

aggregation occurs when added in β-agonists (Fig. 6B and C). These results clearly indicate that the addition of trace β-agonists could readily lead to the aggregation of AuNPs.

Quantitative analysis was investigated by adding different concentrations of β-agonists into MA–AuNPs and monitoring the max absorption by UV–vis spectra. We obtained a linear correlation existed between the absorbance ratio ($A_{670\ nm}/A_{520\ nm}$) and β-agonists concentration ranging from 1×10^{-10} to 5×10^{-7} mol/L with a correlation coefficient of 0.995 for ractopamine, and 1×10^{-10} to 1×10^{-5} mol/L with a correlation coefficient of 0.996 for salbutamol (Fig. 7). The detection limit of these β-agonists is as low as 1×10^{-11} mol/L ($S/N=3$). To the best of our knowledge, the detection limit is the lowest one that has been reported so far in AuNPs-based assays for β-agonists, and our assay has distinctive advantages such as simplicity, rapidness and low cost. To further find the performance of the proposed method, the results compared with those obtained by other methods, as shown in Table 1, we can see that the proposed method exhibited higher sensitivity with lower detection limit and wider linear range.

3.6. Selectivity of MA–AuNPs

Some common potentially interfering substances were investigated to test the selectivity of this system for β-agonists. To indicate the selectivity of MA–AuNPs, interferents of the same concentration with 1×10^{-6} mol/L were added into the solution of AuNPs. As shown in Fig. 8, no obvious change was obtained in the presence of glycine, L-glutamic acid, L-tyrosine, L-arginine, vitamin C, glucose, Ca^{2+}, Mg^{2+} and Na^{+}. The results demonstrated that this sensing system is specific to β-agonists ions. The possible mechanism may be β-agonists have plenty of –OH and –NH– groups to form hydrogen bonding with the melamine, which is similar with the reported literatures [26]. Therefore, the melamine capped AuNPs showed an excellent recognition of β-agonists.

Table 2
The recovery of ractopamine and salbutamol under different concentrations in real samples.

	Sample	Added (mol/L)	Found (mol/L) mean ± SD	Recovery (%)
Ractopamine	1	1×10^{-7}	$9.35\times10^{-8}\pm0.0048$	93.5
	2	5×10^{-7}	$4.62\times10^{-7}\pm0.0021$	92.4
Salbutamol	1	5×10^{-6}	$4.84\times10^{-6}\pm0.0069$	96.8
	2	1×10^{-5}	$10.83\times10^{-6}\pm0.0083$	108.3

3.7. Real sample analysis

To validate the applicability of proposed sensing strategy for the analysis of real swine feed samples, standard solution of ractopamine and salbutamol was added into extracting solution, and then detected utilizing MA–AuNPs based colorimetric method. The results were shown in Table 2, suggesting that MA–AuNPs probe is a practical tool for the determination of β-agonists in swine feed samples.

4. Conclusions

In summary, a novel colorimetric sensor was developed for the highly sensitive and selective detection of β-agonists. The functionalized Au nanoparticles were aggregated due to the hydrogen-bonding interaction between MA and β-agonists. Thus the concentration of the β-agonists could be determined with a UV–vis spectrometer or the naked eyes. The coexisting substances including common amine acid and metal ions did not affect the determination of β-agonists. Particularly, the proposed method could be successfully applied to the determination of ractopamine and salbutamol in real swine feed samples with high recoveries from 92.4% to 108.3%.

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