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Unmodified gold nanoparticles as a simple colorimetric probe for ramoplanin detection

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ABSTRACT

In this paper unmodified gold nanoparticles (AuNPs) were used as a sensing element to detect ramoplamin. Detection relies on the fact that the dispersed AuNPs solution is red due to the intense surface plasmon absorption band at 530 nm whereas the AuNPs solution in the presence of ramoplanin is blue. Upon aggregation, there is a significant change in absorbance intensity at 620 nm. Based on the aggregation of AuNPs induced by ramoplanin, a simple colorimetric method was developed for determining the of ramoplanin concentration. Experimental conditions influencing the analytical performance such as particle size, amount of AuNPs, incubation time and pH were evaluated. Under the optimized experimental conditions, this method could detect ramoplanin in a linear range from 0.30 to 1.30 ppm with a detection limit of 0.01 ppm and exhibited good reproducibility, selectivity and recovery. Analysis time of this assay was only 2 min. To investigate its potential applicability, this assay was successfully applied for the determination of ramoplanin in urine samples without costly instruments.

1. Introduction

Ramoplanin is a novel antibiotic, first disclosed in 1984 [1]. Originally the ramoplanin complex was shown to consist of a mixture of three related compounds, ramoplanin A1–A3, of which ramoplanin A2 is the most abundant. Fig. 1 shows the structure of ramoplanin A2 which is composed of a depsipeptide core structure containing 17 amino acids [2]. It is highly effective against several drug-resistant gram-positive bacteria, including vancomycin-resistant enterococcus faecium (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA), by blocking peptidoglycan synthesis via lipid II [2]. Although ramoplanin is not absorbed through the gastrointestinal tract, detecting ramoplanin is necessary to monitor its levels in plasma and urine [3].

Several methods have been reported for the detection of this new macromolecular drug in real samples such as spectrometry [6], LC–MS [4] and HPLC–UV [3,5]. Recently, a high-performance liquid chromatography technique was used to detect ramoplanin in human urine [3]. However, this method involves long analysis time, complicated operations and high cost. Therefore, a simple, cost-effective, rapid and sensitive method is required for ramoplanin detection.

Gold nanoparticles (AuNPs) exhibit surface plasmon resonance (SPR) due to the collective oscillation of electrons at their surface [6]. The resonance frequency of this SPR strongly depends on the size, shape, dielectric properties and local environment of the AuNPs [7,8]. Thus, any changes to the environment of these AuNPs including surface modification, refractive index and aggregation leads to colorimetric change [9]. An optical probe, AuNPs have been extensively applied in several analytical and biomedical researches for detection of DNA [10], organophosphates [11], mercury(II) [12,13], kanamycin [14] and protein [15] detection. Since the colorimetric optical assays provided simplicity, low cost, time saving and no requirement of sophisticated equipments. Therefore, this paper describes the development of a new method for ramoplanin detection based on colorimetric assay. To the best of our knowledge, there has been no report for detection of ramoplanin using colorimetric assay. This method is the first example of using unmodified AuNPs as a colorimetric probe with a simple and sensitive method for the detection of ramoplanin in real samples. In principle, the present method is based on a color change due to unmodified AuNPs aggregation induced by ramoplanin via high affinity binding between amino groups of ramoplanin and AuNPs (Scheme 1). The aggregation of AuNPs leads to the visual change in color from wine red to purple to blue and results in the formation of a new absorption band at longer wavelengths [16–21]. Several analysis conditions were optimized to achieve a highly sensitive method for the detection of ramoplanin. The proposed method was evaluated by determining the ramoplanin concentration in urine samples.

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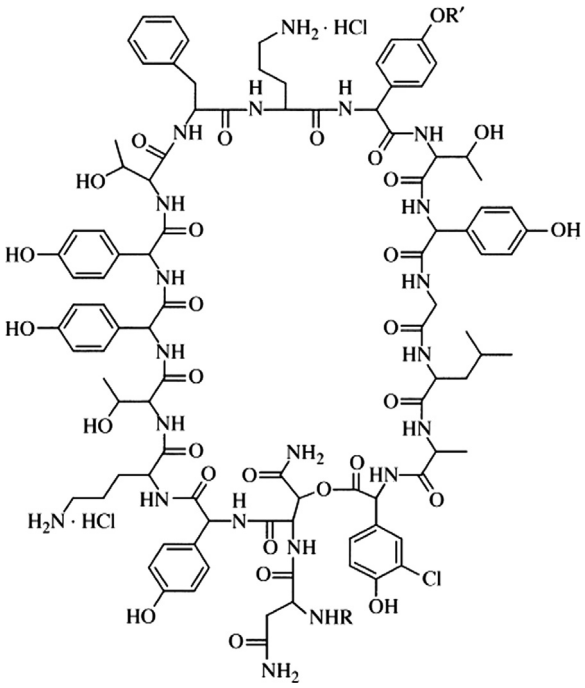
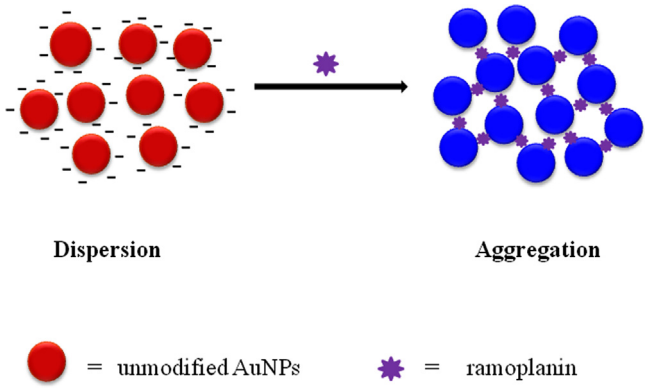


Fig. 1. The structure of ramoplanin.



Scheme 1. Showed the concept for this proposed method based on aggregation of unmodified AuNPs for determination of ramoplanin.

2. Experimental

2.1. Reagents

Hydrogentetrachloroaurate(III) ($HAuCl_4$) sodium borohydride, trisodium citrate and ramoplanin were obtained from Sigma. All other chemicals were of analytical grade and all the solutions were prepared using distilled water.

2.2. Instrument

UV–visible absorption spectra were recorded on a model UV 1601 UV/vis spectrophotometer (Shimadzu). The sizes of dispersed and aggregated AuNPs were measured using dynamic light scattering (DLS) (N5 Submicrometer particle size analyzer, USA).

2.3. Preparation of gold nanoparticles

The AuNPs were prepared via a modified previously reported method [22]. Briefly, 153 μl of 0.3 mM $HAuCl_4$ was added into 500 ml of 0.38 mM trisodium citate under stirring. Then 200 μl of freshly prepared 0.125 M $NaBH_4$ was rapidly added into the above aqueous solution under vigorous stirring. The resulting a purple–red colloidal solution was further stirred for 30 min and then was left undisturbed overnight. The resulting AuNPs was characterized with UV–vis spectroscopy and DLS.

2.4. Detection of ramoplanin using unmodified gold nanoparticle aggregation

For ramoplanin detection, different concentrations of ramoplanin (100 μl) were added into the mixture solution of AuNPs (1800 μl) and distilled water (100 μl). This mixture was then incubated at room temperature for 2 min. UV/vis absorption spectra of the mixture were recorded immediately. The calibration curve for this assay was made by measuring the ratio of UV/vis absorbance between 620 and 530 nm (A_{620}/A_{530}) versus the different concentrations of ramoplanin.

3. Results and discussion

3.1. Principle of ramoplanin detection using unmodified AuNPs aggregation

Scheme 1 shows the concept for this proposed method based on aggregation of AuNPs. The synthesized AuNPs with an average size of 13 nm were estimated from DLS and the concentration of 9×10^{-5} mM. The zeta potential of AuNPs was −41 mV. This result indicated that the AuNPs provided a negative charge. In the absence of ramoplanin, the AuNPs in aqueous solution can be stabilized by negative charge resulting from citrate anion as their repulsion prevented the AuNPs from causing them to aggregate. The AuNPs are readily bound to molecules containing the amine group [23,24]. As ramoplanin contains electron-rich N atom, strong coordination interaction between the N atom and AuNPs, thereby inducing rapid aggregation of the unmodified AuNPs. This results in a dramatic color change from the characteristic wine-red to purple to blue.

3.2. Colorimetric and spectral characteristics for ramoplanin detection

Fig. 2 shows the typical color of unmodified AuNPs which when dispersed in aqueous solution, reveals a wine red color in the absence of ramoplanin. On addition of ramoplanin to the AuNPs, the color changes from wine-red to purple to blue depending on ramoplanin concentration. The observed color change is due to the red shifted absorption band of the functionalized gold nanoparticles upon aggregation according to the Mie theory [25]. As shown in Fig. 2, the original absorption of AuNPs is 530 nm but addition

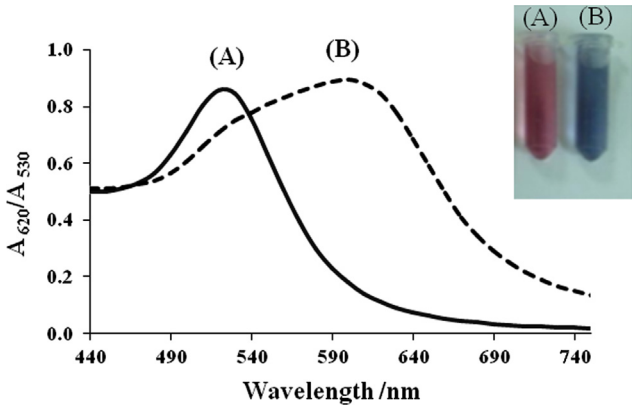


Fig. 2. UV–vis spectra of the absence (A) and presence of 1.00 ppm ramoplanin (B).