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Unmodified gold nanoparticles as a simple colorimetric probe for ramoplanin detection

Siriwan Teepoo*, Phongnarin Chumsaeng, Khwankhao Palasak, Natvara Bousod, Naree Mhadbamrung, Phorntip Sae-lim

Department of Chemistry, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathumthani 12110, Thailand

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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.

* Corresponding author. Tel.: +66 02 5493535; fax: +66 02 5493526.
E-mail address: siriwan@mail.rmutt.ac.th (S. Teepoo).

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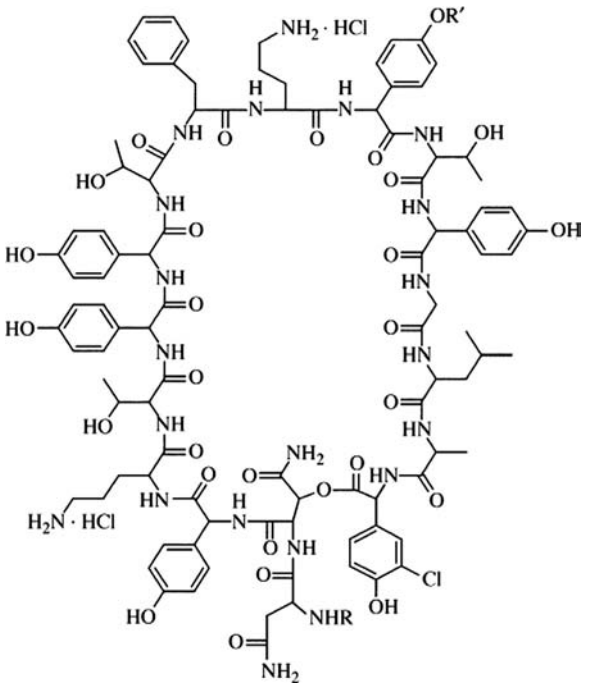
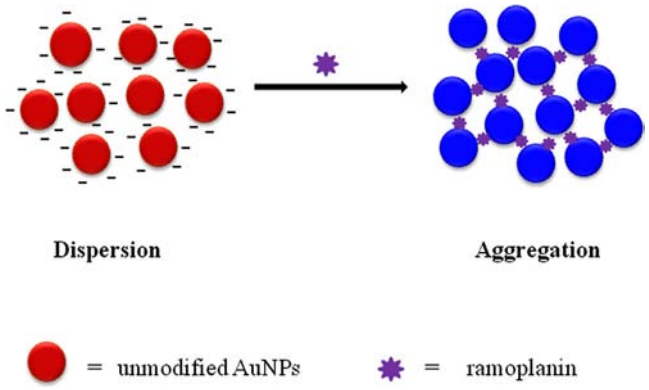


Fig. 1. The structure of ramoplanin.



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

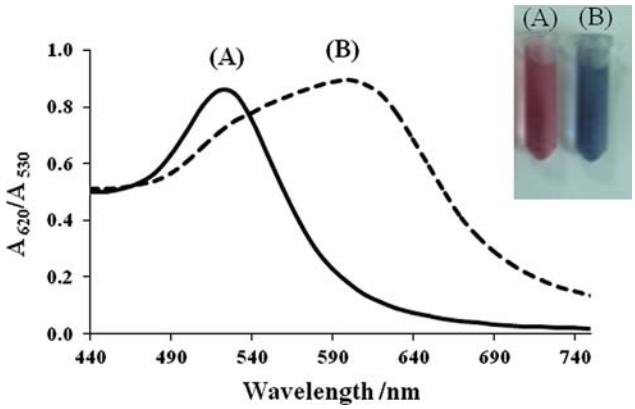


Fig. 2. UV–vis spectra of the absence (A) and presence of 1.00 ppm ramoplanin (B).

of ramoplanin caused a red shift of the surface plasmon band of AuNPs in UV–visible spectrum at longer wavelength (620 nm). The result of UV–visible spectrum corresponds to color change of AuNPs solution. Therefore, the absorbance ratio at these two signature wavelengths (A_{620}/A_{530}) was used to plot against the concentration of ramoplanin for quantitative ramoplanin analysis by using unmodified AuNPs as a colorimetric probe.

3.3. Optimization of experimental parameters

To achieve a highly sensitive assay, conditions such as particle size, the amount of AuNPs solution, incubation time and pH were studied.

Firstly, the particle size of AuNPs was studied by using two different sizes (13 nm and 23 nm). The experimental results show that 13 nm AuNPs provide more sensitive ramoplanin detection compared with larger AuNPs (23 nm) because the surface area of AuNPs increases with the decrease of particles size resulting in better binding with ramoplanin. Thus, 13 nm AuNPs were selected for all experiments.

The effect of the volume of AuNPs on the aggregation induced by ramoplanin was examined in the range of 1000–1900 μl. When the volume was low, the concentration of AuNPs was insufficient to complex with the highest of ramoplanin. On the other hand, when the volume of AuNPs greater than 1800 μl, ramoplanin with low concentration cannot be detected. A volume of 1800 μl of AuNPs was found to give the highest sensitivity.

The interaction between AuNPs and ramoplanin aggregates at different incubation times was studied from 1 to 5 min. When increasing incubation time from 1 to 2 min a pronounced increase in absorbance change and reached the plateau at 2–5 min (Fig. 3A). Thus, 2 min was chosen for this work.

The pH of the reaction medium affects the aggregation of AuNPs [26]. Therefore, the effect of solution pH was optimized by using acetate buffer over the range from 2.0 to 8.0 with a fixed ramoplanin concentration at 0.75 ppm. The results are shown in Fig. 3B. It can be seen that in strong basic media, the absorbance was very low, whereas at strong acidic media the high absorbance was obtained. This is probably due to the fact that in acidic media, amino groups of ramoplanin had a strong positive charge and usefully attach to the negative charge of AuNPs resulting in more aggregation of AuNPs. However, at low pH the solution medium had strong acidic media amino groups of ramoplanin which can be hydrolyzed and then could not induce the aggregation of AuNPs [27]. Thus, the pH of the solution was chosen as 4.0.

3.4. Analytical performance of the proposed ramoplanin assay

Under the optimized experimental conditions, 13 nm AuNPs, 1800 μl of AuNPs, 2 min of incubation time and pH 4.0 of media solution. With the ramoplanin concentration ranging from 0.00 to 2.00 ppm, the color varied as a function of the ramoplanin concentration (Fig. 4). To further confirm the intensity of the AuNPs aggregation, the dynamic light scattering was used. Fig. 5A–F shows the average diameter of AuNPs in the presence of different concentrations of ramoplanin. When no ramoplanin is present, the unmodified AuNPs are well dispersed with a diameter of 13 nm (Fig. 5A). After the addition of ramoplanin, the diameter of the unmodified AuNPs increased from 13 to 1064 nm (Fig. 5B–F). This result is completely in agreement with expectations of the formation of unmodified AuNPs with ramoplanin. The relationship between ramoplanin concentration and the absorption ratio is shown in Fig. 6. The linear regression equation for the ramoplanin in the range of 0.30–1.30 ppm was $Y=0.975X-0.193$ and the correlation coefficient was 0.997. A limit of detection of 0.01 ppm was estimated using three times the standard derivation in blank solution ($n=10$). The limit of quantitation of 0.30 M was determined within acceptable precision (5% of RSD) and accuracy (102% of recovery).

The precision was estimated by successively determining the response of 0.75 ppm of ramoplanin seven times. The reproducibility was acceptable with a relative standard deviation (RSD) of 2%.

In order to study the reproducibility of the assay, seven independently prepared assays were tested by the measurement of 0.75 ppm ramoplanin and the RSD was found to be 5%. These experimental results demonstrate a good reproducibility of the proposed assay.

The recovery of the method was determined by the analysis of spiked urine samples with three different additions of ramoplanin standard solution. The recovery was found to be 96–107%.

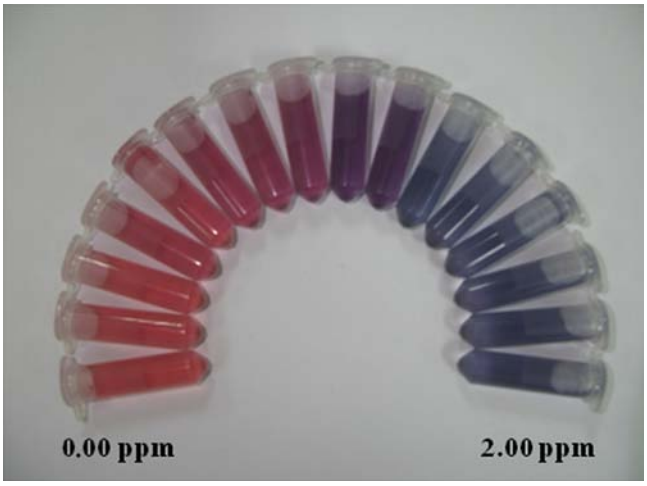


Fig. 4. Visual color changes of AuNPs upon the addition of ramoplanin at different concentrations ranging from 0.00 to 2.00 ppm.

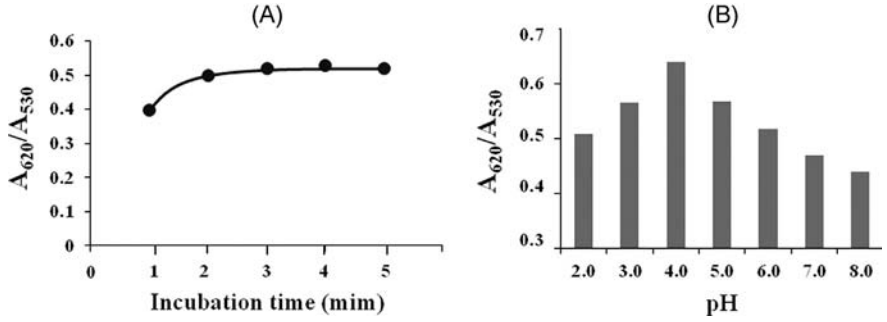


Fig. 3. Effect of incubation time (A) and effect of media pH on the absorption ratio (B).

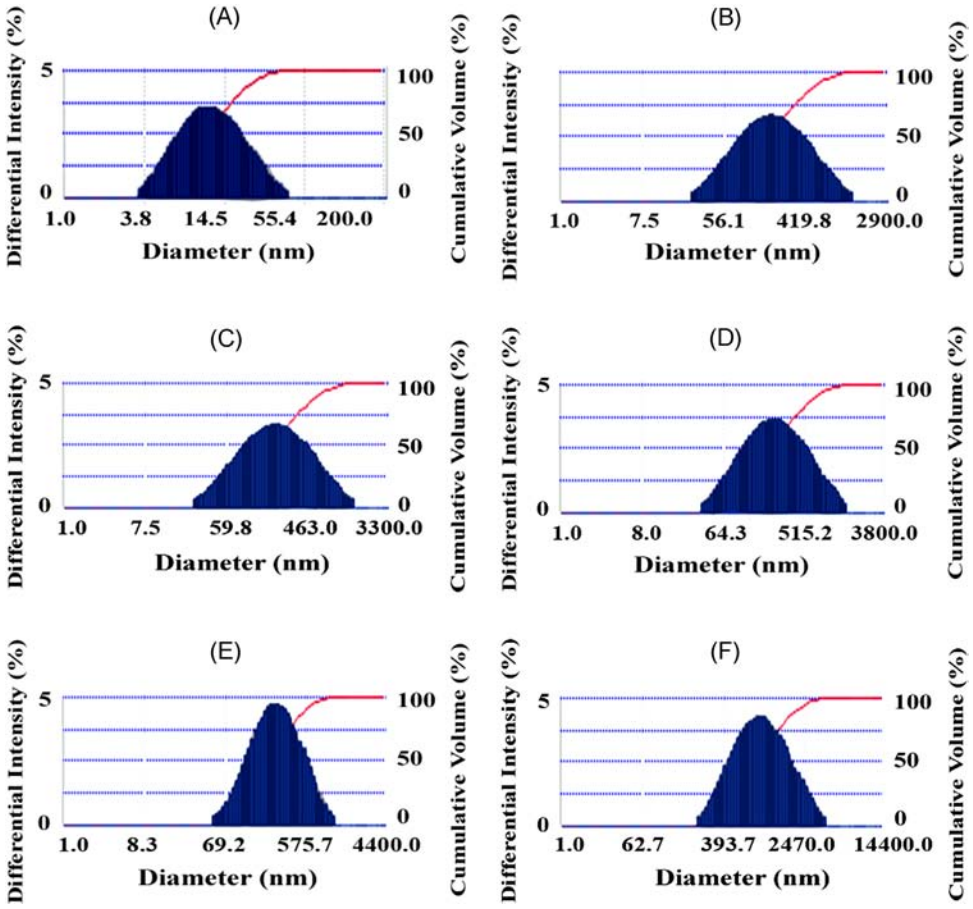


Fig. 5. DLS results of AuNPs (A), AuNPs in the presence of 0.30 ppm (B), 0.50 ppm (C), 0.70 ppm (D) and 0.90 ppm ramoplanin (F).

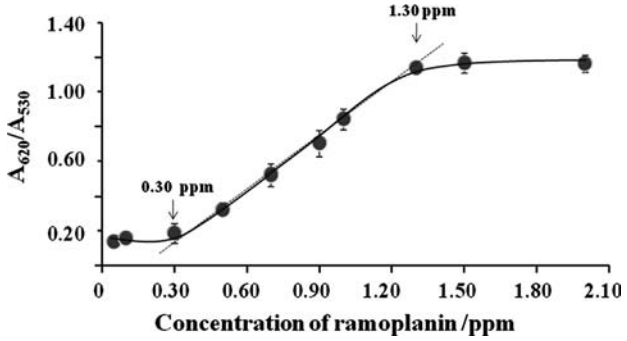


Fig. 6. The calibration curve of ramoplanin detection under optimum conditions.

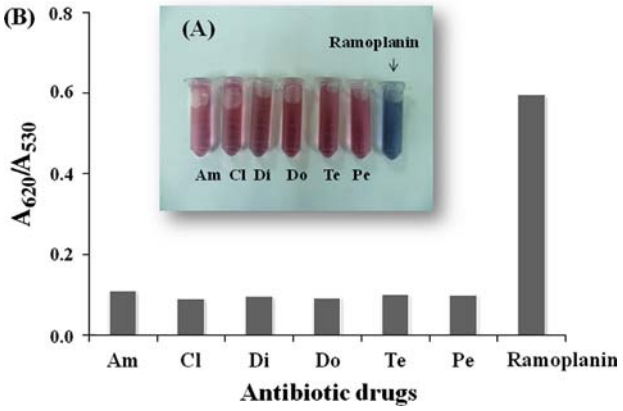


Fig. 7. Selectivity study of this assay. Color change of the solution in the presence of various antibiotic drugs including amoxicillin (Am), cloxacillin (Cl), dicloxacillin (Di), doxycyclin (Do), tetracycline (Te) and penicillin (Pe) at concentrations of 0.75 ppm (A) and the change of the absorption bands at 620 nm for different antibiotic drugs compared with ramoplanin at same concentration (B).

The selectivity of the assay for ramoplanin was evaluated by monitoring the absorption ratio at A_{620}/A_{530} in the presence of other antibiotic drugs including amoxicillin, cloxacillin, dicloxacillin, doxycyclin, tetracycline and penicillin comparing with ramoplanin to induce AuNPs aggregation. As shown in Fig. 7, the absorption ratio and color of ramoplanin-AuNPs exhibited significant change. No significant color change was observed by the addition of antibiotic drug interferents. These indicate that through color observation, the antibiotic drugs mentioned above cannot bring interferences to ramoplanin detection by using unmodified AuNPs. Moreover, the selectivity of this assay was evaluated in the presence of major metabolites (e.g. ascorbic acid, uric acid, Ca^{2+}, NO_3^-) that may exist in urine samples. The results found that all substances did not interfere. Therefore, our assay approach has a high specificity to ramoplanin.

3.5. Applicability to urine samples

In order to evaluate the potential application of unmodified AuNPs in detecting ramoplanin, we tested this proposed assay with urine samples. Five urine samples were analyzed with a proposed method and the results were compared with UV spectrometry at wavelength 270 nm. Ramoplanin was not found by both methods because there were no ramoplanin in urine samples. Then these samples were analyzed with a standard addition method with adding standard of ramoplanin solution at

Table 1
The determination of ramoplanin in urine samples spiked with different concentrations of ramoplanin standard.

Sample	Added (ppm)	Proposed method		UV spectrometry	
		Found[a] (ppm)	Recovery (%)	Found[a] (ppm)	Recovery (%)
Urine-1	0.30	0.32 ± 0.12	107	0.32 ± 0.10	106
Urine-2	0.50	0.49 ± 0.03	98	0.49 ± 0.04	98
Urine-3	0.70	0.67 ± 0.09	96	0.59 ± 0.03	85
Urine-4	0.90	0.93 ± 0.11	103	0.93 ± 0.10	104
Urine-5	1.00	1.01 ± 0.16	101	1.05 ± 0.13	105

[a] Mean ± standard deviation ($n=3$).

different levels and the results were listed in Table 1. It can be seen that the recoveries of this assay vary from 96% to 107%, indicating that the proposed assay has promising feasibility for rapid and sensitive ramoplanin detection in real samples.

4. Conclusion

In this work, we have reported a new assay for ramoplanin based on the fact that ramoplanin can induce the aggregation of AuNPs resulting in red to purple to blue color change which can be easily measured with a common spectrophotometer. The present assay shows several advantages. Firstly, the assay is highly sensitive of ramoplanin analysis with a low detection limit of 0.01 ppm. Secondly, the detection of ramoplanin is completed within only 2 min. Thirdly, this assay exhibits excellent selectivity for ramoplanin over other antibiodic drugs. Finally, the proposed method does not require sophisticated equipment comparing with other common methods. Based on the above reasons, we conclude that our method is simple, fast, sensitive and selective for monitoring ramoplanin concentration by using unmodified AuNPs as a colorimetric probe.

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