RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

High specific determination of gentamicin by induced collapse of Au-lipid capsule

Qianjin Liu[§a,b,c,] , Haibo Mu[§a], Chunli Sun[c] and Jinyou Duan*[a]

[a] College of science, Northwest A&F University, Yangling 712100, Shaanxi, China

[b] Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Kunming, Yunnan, China

[c] Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, China

Correspondence:

Jinyou Duan, Ph.D

Tel.: +86 29 87092226;

E-mail: jduan@nwsuaf.edu.cn

[§] These authors contributed equally to this work

Abstract

Residues of gentamicin in food pose threat to human health. Determination of gentamicin residues relies largely on adequate analytical techniques. Herein, a simple and high specific colorimetric method for the effective detection of this aminoglycoside antibiotic in milk based on gentamicin-induced collapse of Au-lipid capsule is first proposed. The strong interaction between gentamicin and phosphatidylcholine resulted in the collapse of Au-lipid capsule and consequently, the color of AuNPs changed from wine red to blue. The concentration of gentamicin could be determined with naked eye or a UV–vis spectrometer. Results showed that the absorption ratio (A_{664}/A_{531}) was liner with the gentamicin concentration in the range of 0 to 0.2 μM with a linear correlation coefficient of 0.99. The detection limit was 7.4 nM. The coexisting substances including L-arginine, guanidine hydrochloride, Tween-20, ammonium hydroxide, sodium chloride, potassium chloride, calcium chloride, glucose, and other common antibiotics such as streptomycin, amikacin, kanamycin, chloramphenicol, tetracycline, ampicillin, carbenicillin did not interfere with the determination of gentamicin in this method. Furthermore, the established method was successfully applied to qualitative and quantitative analysis of gentamicin in the pretreated milk products.

Keywords: Au-lipid capsule; Gentamicin; Collapse; Specific

1. Introduction

Gentamicin is an aminoglycoside antibiotic, which is used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms.[1, 2] This antibiotic has been widely used not only as an antibacterial drug in human therapy, but also as a veterinary drug in animal husbandry and a crop-protection agent in agriculture.[3, 4] However, gentamicin shows comparatively narrow safety margin and may cause many side effects such as loss of hearing, toxicity to kidneys, and allergic reactions to drugs.[5] Also, the residual amount of gentamicin found in the environments may also lead to antibiotic resistance from the pathogenic bacterial strains, which will pose a serious threat to human health.[6] Apparently, it is of great importance to establish efficient, accurate and economical methods for the detection of genamicin residue in environmental media.

Several methods have been designed for the determination of residual antibiotics including genamicin in environments. Various immunoassays, such as enzyme linked immunosorbent assay (ELISA), fluorescence immunoassay (FIA), radioimmunoassay (RIA) and immunochromatographic assay (ICA) have been employed for the detection of antibiotics residues.[7-12] However, due to the cross-reactions with complicated compounds in food, immunoassays are susceptible to be interfered in real sample analysis.[13] High-performance liquid chromatography (HPLC) is another high-sensitive method which can provide reliable results. However, owing to the lack of chromophore group, post-column derivatization and fluorescence detection are required for trace level detection of gentamicin.[14-16] Liquid chromatography–mass

spectrometry (LC–MS) is also employed for the detection of genamicin and other antibiotics with excellent performance,[17, 18] while the complicated sample preparation and high cost restricts its applications.

In recent years, AuNPs-based colorimetric sensors have been proven as a versatile analytical tool with high sensitivity, due to their unique properties such as color, biocompatibility, stability and distance-dependent surface plasmon resonance (SPR) absorption.[19, 20] Liposome is an artificially-prepared spherical vesicle composed of a lamellar phase lipid bilayer and this unique structure inherently provides liposome with a powerful capability for modified with AuNPs on its surface. In this study, we found that the collapse of Au-lipid capsule could be specifically induced by gentamicin. Subsequently, the color of AuNPs changed from wine red to blue. We demonstrated that this phenomenon was applied successfully to detect trace amount of gentamicin residue in milk samples effectively.

2. Experimental

2.1. Chemicals and materials

Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium citrate tribasic dihydrate was from Bodi Chemical Factory of Tianjin (Tianjin, China). Phosphatidylcholine (soybean,>98%) containing alkyl chains of 16 carbon atoms was purchased from Aladdin, which is from soybean. Streptomycin sulfate, amikacin, chloramphenicol, tetracycline hydrochloride, kanamycin sulfate, carbenicillin disodium salt, ampicillin sodium salt and gentamicin sulfate are all USP grade and purchased from solarbio (Beijing, China). All other chemicals were of analytical grade as available. Ultrapure water (18.25 MΩ cm), obtained from a water purification system, was used in the whole experiment. All the glassware was cleaned with aqua regia and thoroughly rinsed with ultrapure water before use.

2.2. Instrumentation

The absorption spectra were recorded on an evolution 300 UV-Visible spectrophotometer (Thermo, USA) at room temperature (25°C). Scanning electron microscopy (SEM) measurements were performed on an S-4800 (Hitachi, Japan) at 10 kV, Transmission electron microscopy (TEM) measurements were performed on a HT7700 (Hitachi, Japan) at 80 kV.

2.3. Nanoparticle Synthesis

Au particles were prepared by the citrate reduction of $HAuCl_4$ according to previous report with necessary modifications.[21] Typically, 200 μL 1% $HAuCl_4$ was

added to 20 mL ultrapure water (18.25 MΩ cm) that was brought to boil with vigorous stirring in a round-bottom flask fitted with a reflux condenser. 400 μL 1% trisodium citrate was then added rapidly to the solution, and the mixture was heated under reflux for another 30 min. The solution was cooled to room temperature and stored at 4 °C until further use.

2.4. Preparation of Au-lipid capsule

The phosphatidylcholine liposome was fabricated according to previous report with necessary modifications.[22] The purchased phosphatidylcholine (soybean,>98%) (15 mg) was added into 10 mL ultrapure water (18.25 MΩ cm). Then it was vortexed vigorously to make phosphatidylcholine suspension. The phosphatidylcholine suspension with white color was heated at 60°C for 30 minutes to exceed the phase-transition temperature of the used phosphatidylcholine molecules, and then went through sonication at 25°C for 30 minutes to form the phosphatidylcholine liposomes. The AuNPs colloidal solution and the phosphatidylcholine liposomes aqueous solution were mixed at the volume ratio of 1:1, and then mixed immediately by pipetting to make Au-lipid capsule.

2.5. Characterization of AuNPs and Au-lipid capsule

The size and morphology of AuNPs and Au-lipid capsule were characterized by Hitachi S-4800 field emission scanning electron microscopy and Hitachi H-7700 field transmission electron microscopy, The SEM images were acquired by operating at an accelerating voltage of 10 kV. To obtain high resolution images from the SEM analysis, all samples were deposited on a silicon wafer and allowed to dry 30 min.

Then the excess liquid was absorbed from the edges with filter paper to prevent adhesion formation. Especially the collapse of Au-lipid capsule and aggregate of the AuNPs made by treating the solid supported drying the Au-lipid capsule solution treated by gentamicin.

The TEM samples were prepared by placing a drop of the samples onto a Formvar-coated copper grid. The grid was then stained by placing a drop of 1% phosphotungstic acid on its coated-side for 20 sec. Excess stain on the grid was soaked away by touching a filter paper strip. The grid was then dried under a stream or nitrogen gas. All TEM images were taken under the electron accelerating voltage of 80 kV.

2.6. Sample preparation

The liquid milk bought from local supermarket was pretreated to remove protein and fat.[23] Typically, 1.2 mL of 300 g/L trichloroacetic acid was added into 3.0 mL of the spiked milk samples in a centrifuge tube. After thorough vortex, the mixtures were centrifuged at 10,000 rpm for 10 min, and the supernatant was adjusted to the original volume with trichloroacetic acid again. Finally, the solution was filtered using a syringe and 0.22 μm filter and then used for gentamicin determination.

3. Results and discussion

3.1. The mechanism of the sensing system

To better understand the sensing strategy employed in this study, a schematic diagram for the detection of gentamicin by induced collapse of Au-lipid capsule is outlined in Scheme 1. The AuNPs existed on the outside of the liposome membrane to form an Au-lipid capsule. This was because the amine head groups at the outer layer of the phosphatidylcholine liposome could be capped with citrate-stabilized gold nanoparticles through electrostatic interactions.[24] Liposome contributed to stable dispersion of AuNPs, preventing their aggregation. Gentamicin which carries five amino groups could establish electrostatic interactions with the phosphate group on the surface of phosphatidylcholine liposome. Due to the fluidity of the surface of liposome, it can be observed that the high-affinity interaction of gentamicin with phosphatidylcholine molecules on the liposomal surface and subsequently resulted in liposome collapsing and aggregation.[25] As shown in Scheme 1, when the concentration of gentamicin was less than 0.2 μM, the Au-lipid capsules slightly collapse. When the gentamicin was above 0.2 μM, the Au-lipid capsules thoroughly collapse. The color of the suspension was accordingly changed to violet blue. These changes were easily readout by naked eyes. And subtle differences could be measured by spectrophotometer. Given that the strong binding affinity between phosphatidylcholine and gentamicin makes this method highly specific, this study essentially offers a simple but specific and rapid method for gentamicin detection.

3.2. Characterization of AuNPs and Au-lipid capsule

Fig. 1A showed the surface plasma resonance of AuNPs (a) and Au-lipid capsule (b). In the absence of liposome, AuNPs were wine red and displayed an intense surface plasma band at about 531 nm. While in the presence of liposome, the absorbance of AuNPs at 531 nm slightly decreased, indicating the presence of AuNPs on the outer membrane of liposome surfaces without disturbing the spherical topography. The SEM in Fig. 1B, Fig. S3 and TEM image in Fig. S1 confirmed that AuNPs were present on the outer membrane of liposome surfaces, which was consistent with the previous report.[26]

Also the stability of the Au-lipid capsule was tested. As key factors for most electrostatic reactions, the influence caused by pH and ionic strength of the Au-lipid capsule suspension was tested. The Au-lipid capsule suspension was treated with varied pH or different NaCl concentration for one hour, and then the absorption ratio A_{664}/A_{531} was observed. In this test, the absorption ratio of A_{664}/A_{531} is monitored to represent aggregation level. As shown in Fig. S2A, the absorption ratio of A_{664}/A_{531} was the highest at pH 2, indicating the maximum aggregation level of AuNPs. Generally, AuNPs prepared by using tri-sodium citrate carry negative charges. So the lower pH would weaken the electrostatic interaction between AuNPs and amine head groups at the outer layer of the liposome, and lead to the aggregation of AuNPs. Also the absorption ratio of A_{664}/A_{531} was found to increase with increasing the ionic strength in Fig. S2B.

3.3. Optimization of experimental conditions

3.3.1. The effect of media pH

Fig. 2A illustrated the influence of media pH on the absorption ratio (A_{664}/A_{531}) in the presence of 0.6 μM gentamicin. As shown, at the media pH under weak basic conditions (pH=7–9) or acidic conditions (pH = 5–6), the probe did not show good response. The absorption ratio (A_{664}/A_{531}) was the highest at pH 5, indicating the maximum aggregation level of AuNPs, which coincided with the change in the solution color (Fig. 2B). Therefore, pH 5 was set as the operational pH for subsequent experiments.

3.3.2. The effect of temperature

Fig. 3 illustrated the influence of temperature on the absorption ratio (A_{664}/A_{531}) in the presence of 0.6 μM gentamicin. It was observed that the temperature had little effect on response of Au-lipid capsule to gentamicin. It might be because the temperature influenced the mobility of the individual lipid molecules. For convenience, all the absorption measurements were performed for subsequent experiments at room temperature (25°C).

3.4. Colorimetric sensing of gentamicin

To demonstrate the performance of the Au-lipid capsule probe, different concentrations of gentamicin ranging from 0 to 0.8 μM were added to aqueous solution of Au-lipid capsule. Upon addition of increasing concentrations of gentamicin, the color of Au-lipid capsule gradually changed from initially wine red to purple and finally to blue (Fig. 4A). These changes are related to a plasmon coupling effect of AuNPs: the reduction of the distance between AuNPs particles because of aggregation, leading to a strong enhancement of the localized electric field and

increasing refractive indices.[27] Addition of gentamicin induced serious collapse of Au-lipid capsule, leading to the increase of AuNPs aggregation. The aggregation of AuNPs was evidenced by UV–vis spectra shown in Fig. 4B. As expected, with the increase of gentamicin concentration, the surface plasmon resonance at 531 nm decreased, while at the same time, a new absorption band around 664 nm appeared and gradually increased. The corresponding effect was evaluated by comparing the A_{664}/A_{531} values in the presence of different concentrations of gentamicin for quantitative analysis (Fig. 4C). Consistently, the A_{664}/A_{531} increased significantly was observed at the concentration ranging from 0 to 0.4 μM and a slight increase was observed in the concentration ranging from 0.4 to 0.8 μM.

The genamicin-induced collapse of Au-lipid capsule and the aggregation of AuNPs were further confirmed by SEM (Fig. 5). First, the initial AuNPs were well dispersible on the liposome surface to form an Au-lipid capsule (Fig. 5a). However, after adding 0.02 μM of gentamicin, the slight collapse of Au-lipid capsule and the random agglomerate of AuNPs, driven by attraction between the negative charges on the surface of phosphatidylcholine liposome and positive charges on the gentamicin molecules, was observed (Fig. 5b). When the concentration of gentamicin increased up to 0.1 or 0.2 μM, the Au-lipid capsule seriously collapsed and large numbers of AuNPs accumulated (Fig. 5c and 5d).

A good linear correlation existed between the absorption ratio A_{664}/A_{531} of Au-lipid capsule and the concentration of gentamicin in the range of 0 to 0.2 μM with a correlation coefficient of 0.99 (Fig. 6). The detection limit of the proposed method

was 7.4 nM, which was calculated as LOD = 3×(SD/S), where SD is the standard deviation of the response and S is the slope of the calibration curve.

3.5. Specificity of the assay

Specificity is an important aspect to evaluate the performance of a new proposed assay. Thus, it is necessary to explore the selectivity of the proposed assay. The selectivity of the probe for gentamicin was evaluated by monitoring the absorption ratio (A_{664}/A_{531}) in the presence of various bioanalytes in comparison with blank test (Fig. 7A). Firstly, the responses of Au-lipid capsule to gentamicin and to other antibiotic molecules were compared. The absorption ratio (A_{664}/A_{531}) showed little change in the presence of 0.8 μM of other antibiotics including streptomycin, chloramphenicol, tetracycline, amikacin, kanamycin, ampicillin, carbenicillin. This result revealed that the Au-lipid capsule showed no cross-reactivity with those antibiotics above. It was demonstrated that instead of other aminoglycoside antibiotics, there was strongest interaction between genamicin and phosphatidylcholine, due to the positive charge, special conformation and the amphiphilic properties of gentamicin[28]. Second, we evaluated Au-lipid capsule response to molecules carrying positively charged groups such as L-arginine, guanidine hydrochloride, ammonium hydroxide. As a result, Au-lipid capsule showed no response to these molecules. In addition, we also monitored the Au-lipid capsule response to KCl, $CaCl_2$, NaCl, glucose and Tween-20, which might coexist with gentamicin in the environment. The UV-Vis spectra revealed that these molecules did not interfere in gentamicin detection. However, the control results of AuNPs didn't show specific for gentamicin (Fig. 7B).

All of the results showed that the probe of Au-lipid capsule could detect gentamicin with high selectivity.

3.6. Analysis of gentamicin in real samples

To evaluate the practical application of the proposed colorimetric method, the detection of milk sample was carried out by standard addition method according to most relative publications.[29-31] As the Maximum Residue Limits (MRLs) of some aminoglycoside antibiotics in milk are between 0.14 μM and 1.0 μM,[32] we chose 0.05 μM and 0.1μM to study the recoveries of gentamicin. As showed in Table 1, the recoveries of gentamicin were 88.9% and 108.6% with the coefficient of variation less than 10% (n=6), indicating the promising feasibility of this colorimetry for gentamicin quantification. Furthermore, a red-to-blue color change could also be observed upon addition of the milk sample with naked eye (Fig. 8). Therefore, the proposed method could be employed to analyze the antibiotics in pretreated milk samples.

4. Conclusion

In this work, a novel colorimetric sensor was proposed for the highly sensitive and selective detection of gentamicin. The strong electrostatic interaction between gentamicin and phosphatidylcholine rapidly induced the collapse of Au-lipid capsule and consequently, the AuNPs aggregated. As a result, the color of Au-lipid capsule solution changed from red to blue, which could be determined with the naked eye or a UV–vis spectrometer. Parameters that affect the sensitivity and the possible interferential substances of the experiment were investigated. Compared to other traditional detection method for gentamicin (Table S1), the proposed approach

presented satisfactory linear range, low detection limit, short detection time, good accuracy and specificity for the convenient detection of gentamicin. In addition, we found that Au-lipid capsule were suitable to monitor gentamicin in milk samples efficiently, which could be applied as a promising candidate for on-site detection of this antibiotic commonly used.

Acknowledgments

This project was supported by the National Natural Science Foundation of China (NSFC) [Grant 31270860] and Program for New Century Excellent Talents in University (NCET-13-0480) and Yangling Agricultural Hi-tech Industries Demonstration Zone (2014NY-35). We are particularly grateful to Jianlong Wang, for his good suggestions.

References

1. M. J. Weinstein, G. M. Luedemann, E. M. Oden and G. H. Wagman, *Antimicrobial agents and chemotherapy*, 1963, 161, 1.
2. R. F. Moulds and M. S. Jeyasingham, *Australian Prescriber*, 2010, 33, 134-135.
3. S. A. McEwen and P. J. Fedorka-Cray, *Clinical Infectious Diseases*, 2002, 34, S93-S106.
4. A. K. Vidaver, *Clinical Infectious Diseases*, 2002, 34, S107-S110.
5. R. Oertel, V. Neumeister and W. Kirch, *Journal of Chromatography A*, 2004, 1058, 197-201.
6. D. A. Tadesse, S. Zhao, E. Tong, S. Ayers, A. Singh, M. J. Bartholomew and P. F. McDermott, *Emerg Infect Dis*, 2012, 18, 741-749.
7. N. Pastor-Navarro, Á. Maquieira and R. Puchades, *Analytical and bioanalytical chemistry*, 2009, 395, 907-920.
8. B. G. Knecht, A. Strasser, R. Dietrich, E. Märtlbauer, R. Niessner and M. G. Weller, *Analytical Chemistry*, 2004, 76, 646-654.
9. S. Rebe Raz, M. G. Bremer, W. Haasnoot and W. Norde, *Analytical chemistry*, 2009, 81, 7743-7749.
10. N. Byzova, E. Zvereva, A. Zherdev, S. Eremin, P. Sveshnikov and B. Dzantiev, *Analytica chimica acta*, 2011, 701, 209-217.
11. Y. Bang-Ce, L. Songyang, Z. Peng and L. Xiao-Hong, *Food chemistry*, 2008, 106, 797-803.
12. Y. Chen, Y. Shang, X. Li, X. Wu and X. Xiao, *Food chemistry*, 2008, 108, 304-309.
13. S. Jongberg, V. Orlien, L. H. Skibsted and S. Weigel, *European Food Research and Technology*, 2009, 228, 659-664.
14. P. Viñas, N. Balsalobre and M. Hernández-Córdoba, *Talanta*, 2007, 72, 808-812.
15. P. Edder, A. Cominoli and C. Corvi, *Journal of chromatography A*, 1999, 830, 345-351.
16. A. Posyniak, J. Zmudzki and J. Niedzielska, *Journal of Chromatography A*, 2001, 914, 59-66.
17. K. Vučićević-Prčetić, R. Cservenak and N. Radulović, *Journal of pharmaceutical and biomedical analysis*, 2011, 56, 736-742.
18. S. B. Turnipseed, S. B. Clark, C. M. Karbiwnyk, W. C. Andersen, K. E. Miller and M. R. Madson, *Journal of Chromatography B*, 2009, 877, 1487-1493.
19. S. K. Ghosh and T. Pal, *Chemical Reviews*, 2007, 107, 4797-4862.
20. R. A. Sperling, P. R. Gil, F. Zhang, M. Zanella and W. J. Parak, *Chemical Society Reviews*, 2008, 37, 1896-1908.
21. C.-C. Huang and W.-L. Tseng, *Analyst*, 2009, 134, 1699-1705.
22. C. Tsukada, T. Tsuji, K. Matsuo, H. Nameki, T. Yoshida and S. Yagi, *Journal of Surface Analysis*, 2014, 20, 230-233.
23. Y. Wang, D. Zhang, W. Liu, X. Zhang, S. Yu, T. Liu, W. Zhang, W. Zhu and J. Wang, *Biosensors and Bioelectronics*, 2014, 55, 242-248.
24. S. Chatterjee, M. Krikorian, H. D. Gafney and B. Gersten, *arXiv preprint arXiv:1207.3474*, 2012.
25. F. Van Bambeke, P. M. Tulkens, R. Brasseur and M.-P. Mingeot-Leclercq, *European Journal of Pharmacology: Molecular Pharmacology*, 1995, 289, 321-333.
26. M. Bhuvana and V. Dharuman, *Analyst*, 2014, 139, 2467-2475.
27. M. E. Stewart, C. R. Anderton, L. B. Thompson, J. Maria, S. K. Gray, J. A. Rogers and R. G. Nuzzo, *Chemical reviews*, 2008, 108, 494-521.

28. R. Brasseur, G. Laurent, J. M. Ruysschaert and P. Tulkens, *Biochemical pharmacology*, 1984, 33, 629-637.
29. X. Zhang, H. Zhao, Y. Xue, Z. Wu, Y. Zhang, Y. He, X. Li and Z. Yuan, *Biosensors and Bioelectronics*, 2012, 34, 112-117.
30. Z. Wu, H. Zhao, Y. Xue, Q. Cao, J. Yang, Y. He, X. Li and Z. Yuan, *Biosensors and Bioelectronics*, 2011, 26, 2574-2578.
31. X. Zhang, Y. Zhang, H. Zhao, Y. He, X. Li and Z. Yuan, *Analytica chimica acta*, 2013, 778, 63-69.
32. E. E. Loomans, J. van Wiltenburg, M. Koets and A. van Amerongen, *Journal of agricultural and food chemistry*, 2003, 51, 587-593.

Figure Captions

Scheme 1. Schematic illustration of Au-lipid capsule sensing system for gentamicin detection.

Fig. 1. (A) UV-Vis absorption spectra of AuNPs (a) Au-lipid capsule (b); (B) SEM image of AuNPs (a) and Au-lipid capsule (b).

Fig. 2. (A) Effect of pH value on the absorption ratio (A_{664}/ A_{531}) of Au-lipid capsule with the addition of 0.6 μM gentamicin; (B) Photo of the Au-lipid capsule with the addition of 0.6 μM gentamicin under different pH conditions (pH = 5-9). Data are from three separate experiments. The data are expressed as means ± SD. Error bars represent standard deviation.

Fig. 3. Absorption ratio A_{664}/A_{531} of Au-lipid capsule (red line) and Au-lipid capsule with 0.6 μM gentamicin (blue line) under different temparatures. These experiments were performed three times with similar results each time. The data are expressed as means ± SD. Error bars represent standard deviation.

Fig. 4. (A) Visual colorimetric change of the Au-lipid capsule solution upon addition of gentamicin with different concentrations; (B) UV-Vis absorption spectra of the Au-lipid capsule upon addition of gentamicin with different concentrations (0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 μM). (C) The plot of ratio A_{664}/A_{531} versus gentamicin concentrations. All experiments were performed in triplicate. The data are expressed as means ± SD. Error bars represent standard deviation.

Fig. 5. SEM characterized Au-lipid capsule aggregation upon addition of gentamicin concentrations up to 0 μM (a), 0.02 μM (b), 0.1 μM (c), 0.2 μM (d).

362 **Fig. 6.** Standard calibration curves of A_{664}/A_{531} against the gentamicin concentrations
363 from 0 to 0.2 μM. All experiments were performed in triplicate. The data are
364 expressed as means ± SD. Error bars represent standard deviation.
365 **Fig. 7.** (A) Absorption ratio A_{664}/A_{531} of Au-lipid capsule in the presence of different
366 analytes in comparison to Au-lipid capsule solution. (B) Absorption ratio A_{664}/A_{531} of
367 AuNPs in the presence of different analytes in comparison to AuNPs solution. Error
368 bars show the standard deviations of measurements taken from three independent
369 experiments.
370 **Fig. 8.** Visual colorimetric change of the optimized Au-lipid capsule probe: (a) with
371 the addition of the extract from blank milk sample; (b) with the addition of the extract
372 containing 0.02μM gentamicin; (c) with the addition of the extract containing 0.05
373 μM gentamicin; (d) with the addition of the extract containing 0.1 μM gentamicin.
374

375

Samples	Added concentration (μM)	Measured concentration (μM)	Recovery (%)	CV (%)
Milk 1	0.05	0.04445	88.9	3.8485
Milk 2	0.1	0.1086	108.6	4.6905

376 **Table 1** Detection of gentamicin levels in spiked milk.

Scheme 1

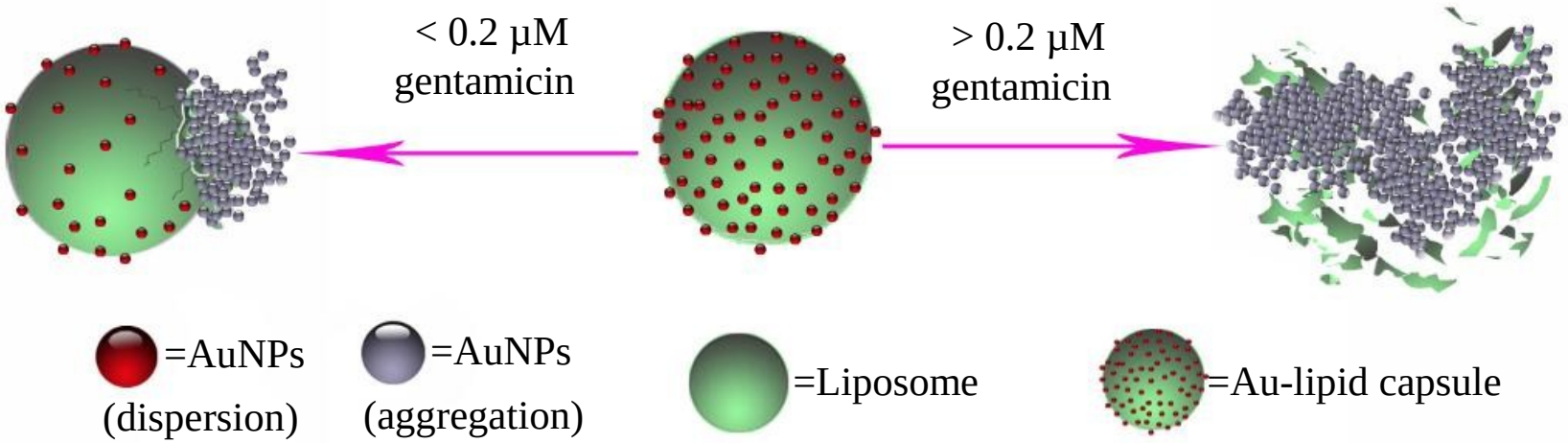
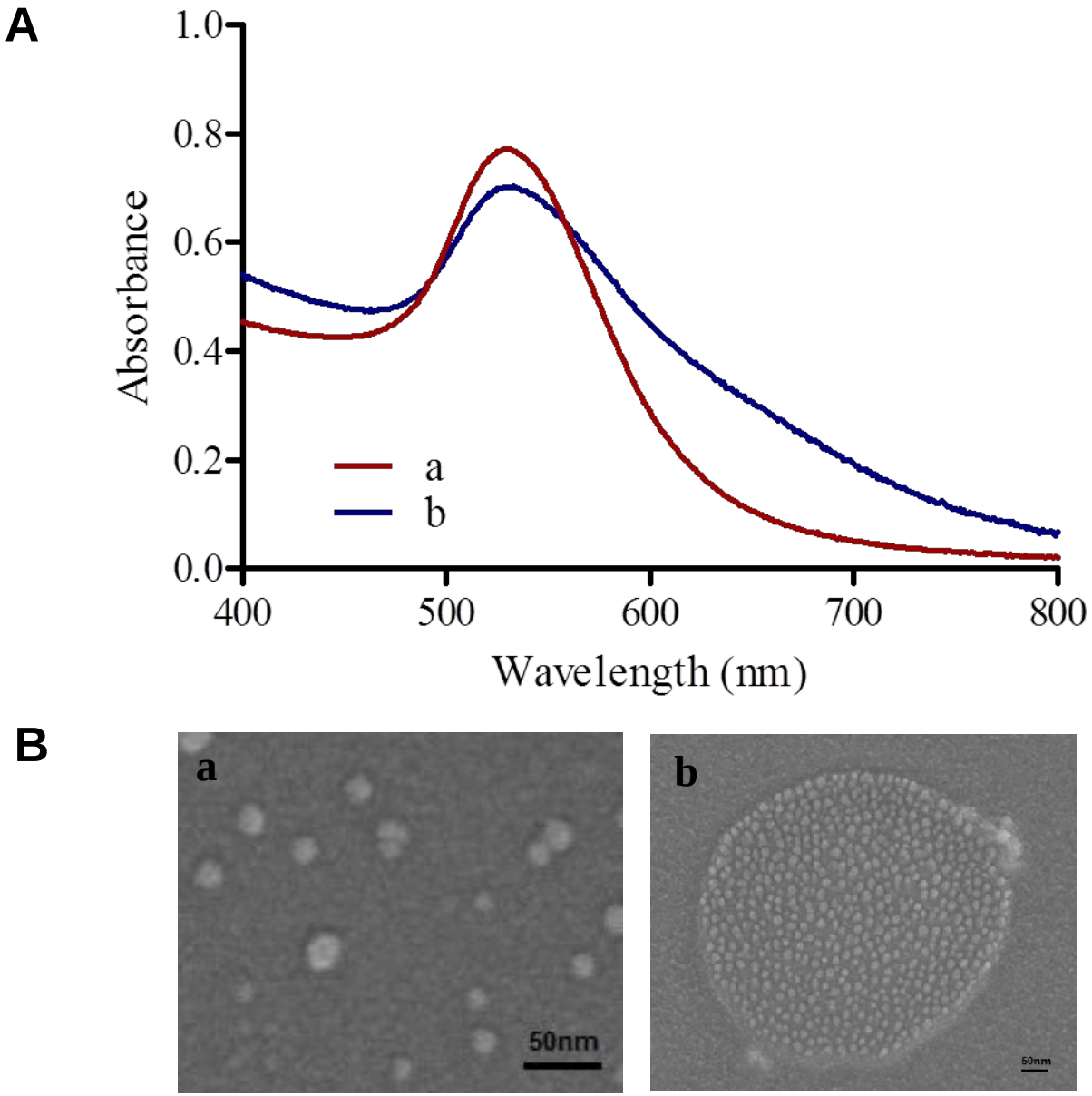


Fig. 1



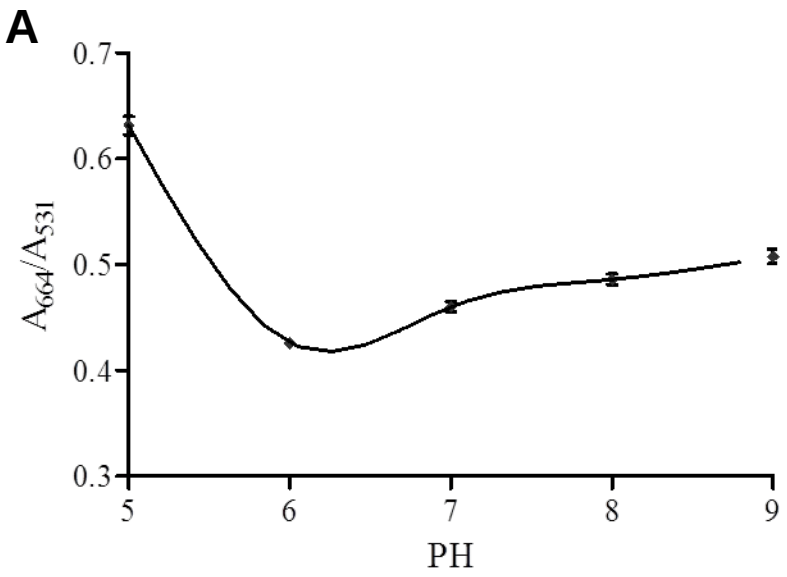
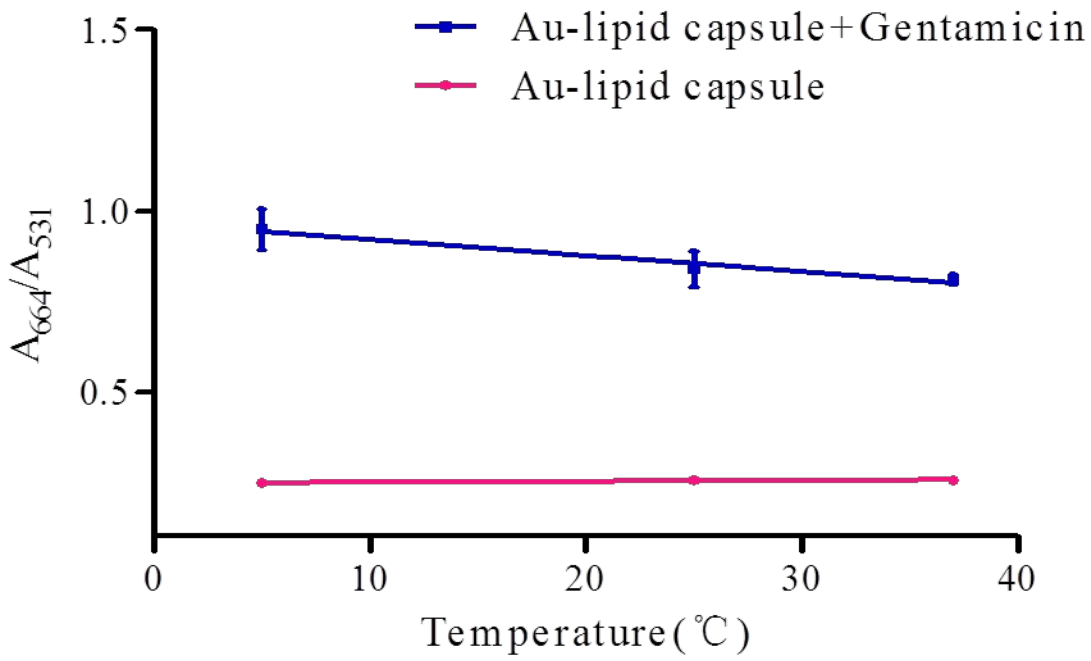
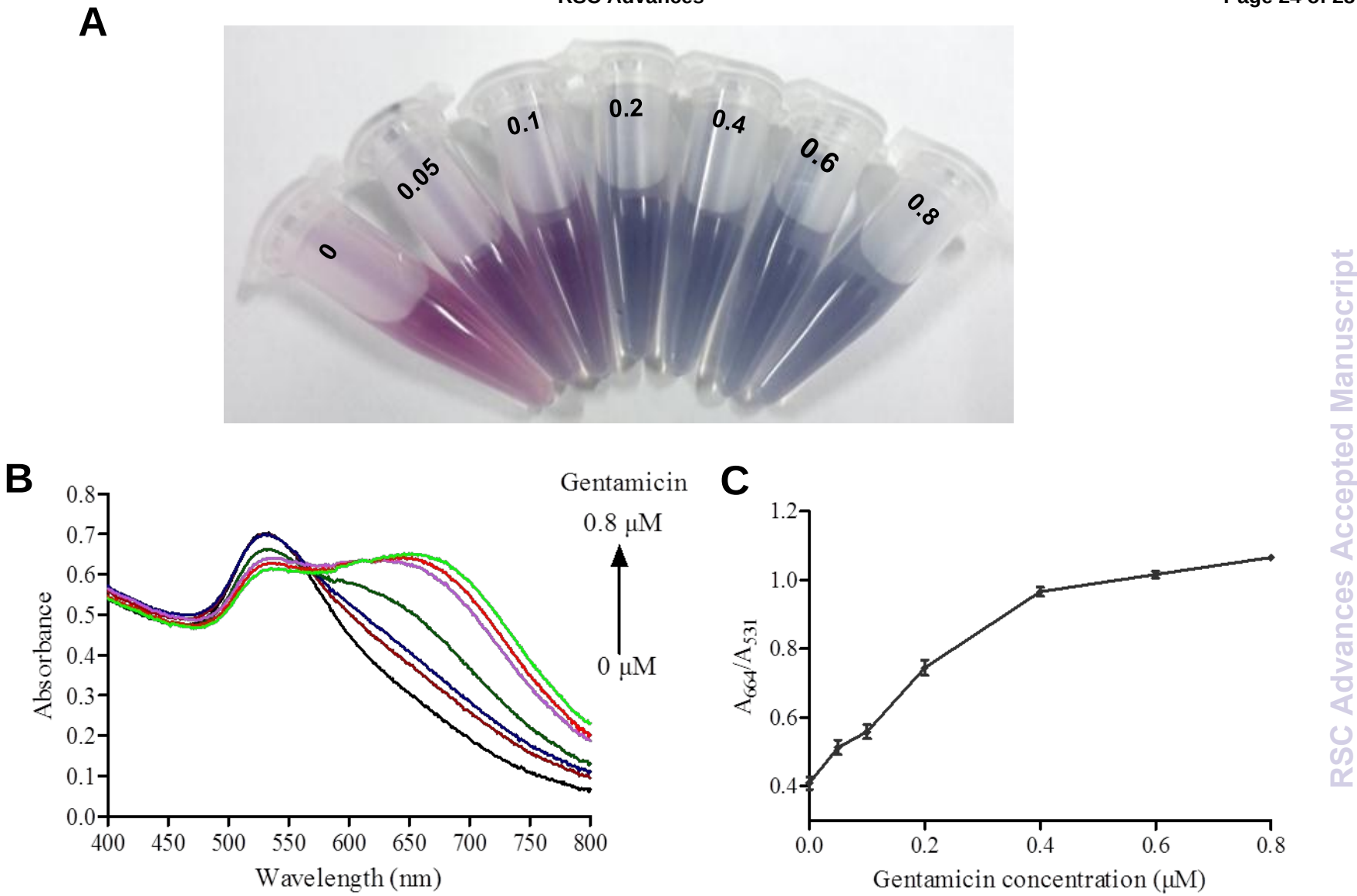
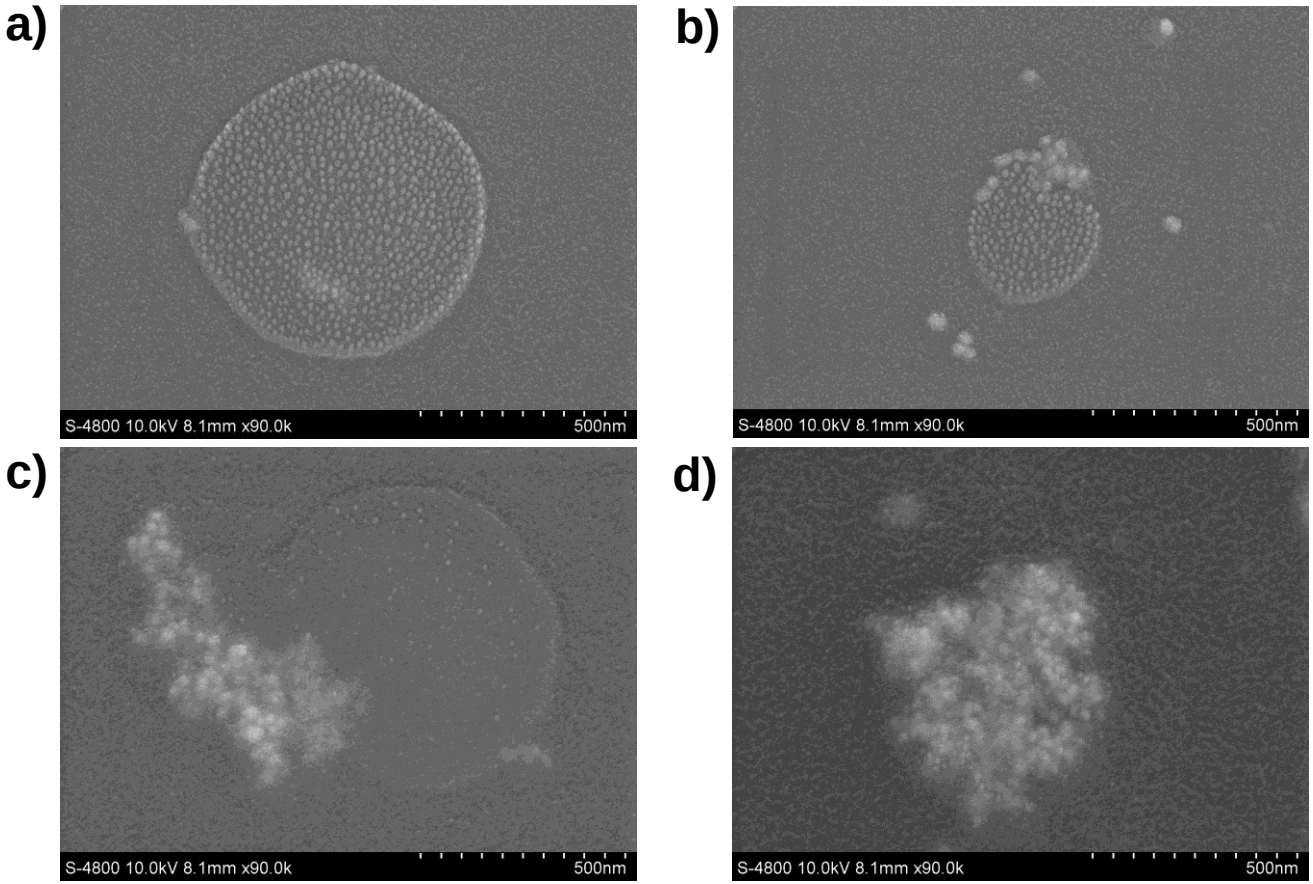


Fig.3







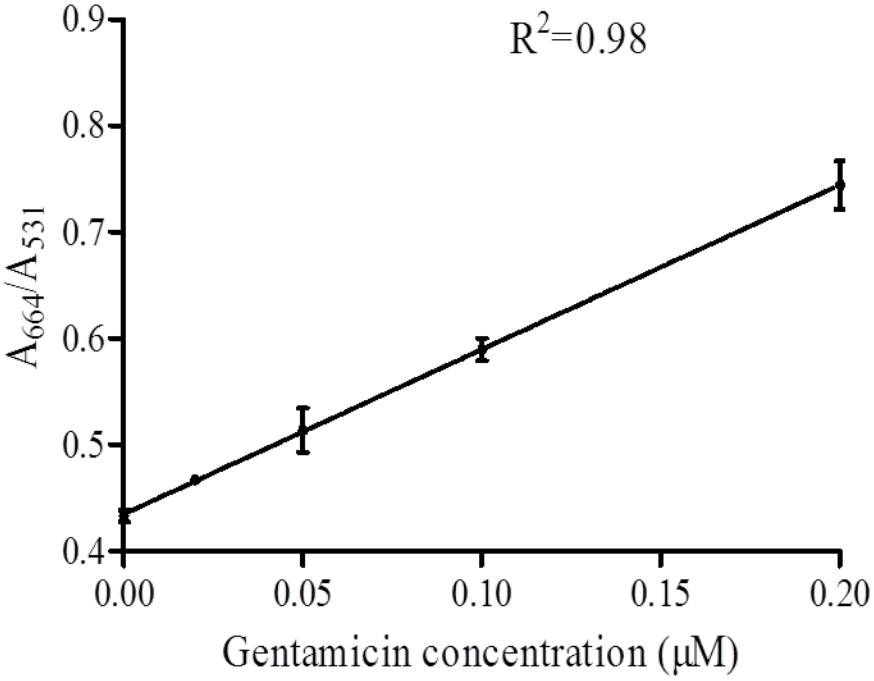


Fig. 7