Research Article

Received: 9 August 2019 Revised: 22 October 2019 Accepted article published: 4 November 2019 Published online in Wiley Online Library: 10 December 2019

(wileyonlinelibrary.com) DOI 10.1002/jsfa.10129

Silver nanoparticle probe for colorimetric detection of aminoglycoside antibiotics: picomolar-level sensitivity toward streptomycin in water, serum, and milk samples

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Abstract

BACKGROUND: The low cost of aminoglycoside (AMG) antibiotics facilitates their excessive use in animal husbandry and the agriculture sector. This scenario has led to the occurrence of residues in the food chain. After several years of AMG use in antibacterial therapy, resistance to streptomycin has begun to appear. Most of the detection methods developed for AMG antibiotics lacks specificity. A broad target specific nanoprobe would be ideal for detecting the entire class of AMGs. A rapid and sensitive method for the detection of AMGs is urgently needed.

RESULTS: Gallic acid-coated silver nanoparticles (AgNPs) were demonstrated as a nanoprobe for the colorimetric detection of AMGs (yellow to orange / red). A linear dynamic range of 50–650 pmol L^{-1} was achieved readily by ratiometric spectrophotometry (A_{560}/A_{400}) with a limit of detection (LOD) as low as 36 pmol L^{-1}. The amine-groups of the AMGs function as molecular linkers, so that electrostatic coupling interactions between neighboring particles drive the formation of AgNP aggregates. The assay can also be applied for the determination of streptomycin residues in serum and milk samples.

CONCLUSION: This study revealed the potential of an AgNP probe for the rapid and cost-effective detection of low-molecular-weight target analytes, such as the AMGs. A ligand-induced aggregation of AgNPs coated with gallic acid was reported to be a rapid and sensitive assay for AMGs. Analysis of streptomycin was demonstrated with excellent picomolar-level sensitivity. Thus, the validated method can find practical applications in the ultrasensitive detection of AMGs in complex and diagnostic settings.

Keywords: AgNP probe; ratiometric assay; picomolar sensitivity; antibiotics residues; food safety

INTRODUCTION

Aminoglycoside (AMG) antibiotics are RNA-binding drugs with a common core structure called the streptamine ring. They bind to the ribosomal subunit, thereby hindering mRNA translation and finally leading to nonsense mutation and rapid cell death. Aminoglycoside antibiotics are known for their broad-spectrum antibacterial action against a range of gram-negative aerobic bacteria.[1] However, the substantial variation between the administered AMG antibiotic dose and variations in the resultant levels in the blood is of great concern.[2] The negative impact of antibiotic residues in food and water has also received worldwide attention due to their abuse in animal husbandry and agricultural practices.[3,4] This global scenario has emerged as a pressing concern owing to the evolving multi-drug resistance observed for bacteria.[5] There is thus an urgent need to develop novel assays with high selectivity and sensitivity for the detection of AMG antibiotics in water and food samples.

Streptomycin is a broad-spectrum AMG antibiotic that was discovered in 1943 from a soil actinomycete, *Streptomyces griseus*.[6] It is effective for gram-negative bacterial treatment and is used not only in controlling human diseases but also in modern agriculture

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veterinary medicine, and other sectors.[7] The presence of streptomycin residues in animal-derived foodstuffs has resulted in serious side effects affecting human health, such as toxicity to the kidneys, loss of hearing, and allergic reactions.[8] Most countries have therefore set limits for streptomycin residues in water and food samples. The European Commission has set a maximum residual limit (MRL) of about 500 and 200 μg kg^{-1} for streptomycin in meat and milk, respectively.[9] Similar criteria have also been adopted by China. Antibiotic-contaminated milk is also known to inhibit starter cultures used in cheese and yogurt production, resulting in economic losses to the dairy industries. Thus, the development and application of nanoprobes,[10] for sensitive and selective detection of streptomycin is in significant demand to ensure human health as well as food quality and safety.

The most well-known method proposed for the detection of antibiotics is a microbial inhibition screening test.[11] However, the sensitivity of such agar-based diffusion methods remains questionable. The residue levels of antibiotics are predominantly detected by high-performance liquid chromatography, liquid chromatography–mass spectrometry, and gas chromatography–mass spectrometry.[12] Enzyme-linked immunosorbent assay, fluorescence immunoassay, and radioimmunoassay have also been used to detect streptomycin residues; however, cross-reactions can prevent efficient determination of the target analyte.[9] Developing new methods based on metal nanoparticles (NPs) for rapid, selective, and sensitive detection of streptomycin and other AMGs therefore remains desirable for on-site monitoring applications.

In general, AMGs are difficult to detect directly due to the lack of a UV-absorbing chromophore. However, AMGs with streptamine rings possessing multiple amino groups can be discriminated from other categories of antibiotics. The broad target analyte specificity of a gallic acid molecular probe bonded with AgNPs would be ideal for the rapid detection of the entire class of AMGs. Colorimetric detection with specificity toward AMGs is proposed here, with quantitative ratiometric measurements of streptomycin levels in the water, serum, and milk samples at ambient temperature (22–24 °C). The limit of detection (LOD) values obtained were far below those of MRL levels of streptomycin in drinking water, milk, and other products. The results showed that the AgNP probe is rapid, selective, and sensitive toward AMGs, and thus could serve as the basis for novel assessment to ensure food safety and human health.

MATERIALS AND METHODS

Chemicals and reagents

Silver nitrate ($AgNO_3$), ampicillin, tetracycline, streptomycin, chlortetracycline, and penicillin G were obtained from Sigma–Aldrich (St. Louis, USA). Oxytetracycline, metacycline, amikacin, and penicillin V were obtained from Cayman (Michigan, USA). Kanamycin, gentamycin, erythromycin, clarithromycin, and azithromycin were obtained from Tokyo Chemical Industry, Tokyo, Japan. Doxycycline, tobramycin, and neomycin were obtained from Alfa Aesar (Tewksbury, USA). Sodium chloride, hydrochloric acid, and sodium hydroxide were obtained from Dae Jung Chemical Co. (Shiheung, South Korea).

Synthesis and purification of AgNPs

Green synthesis of silver nanoparticles (AgNPs) was undertaken according to a reported procedure with minor modifications,[13] using gallic acid, a natural reducing agent, and a stabilizing agent. In brief, 4 mL of gallic acid (12.5 μmol L^{-1}) was added to 0.5 mL of $AgNO_3$ (20 mmol L^{-1}) under stirring, and the total volume of the reaction mixture was adjusted to 10 mL by nanopure water. The AgNP synthesis was initiated by adding a dilute solution of NaOH (0.05 mL, 1 mol L^{-1}) to the aqueous solution mentioned above, which turned from colorless to brown in less than a minute accompanied by a well-defined surface plasmon resonance band at 400 nm. Then, 0.2 mL AgNP aliquots, collected at different time intervals, were diluted by adding 0.8 mL of nanopure water. The average ($n = 3$) absorbance intensity observed at 420 nm was plotted against time to observe the reaction kinetics. The effect of NaOH and gallic acid concentrations on the UV-visible spectrum and the reaction kinetics of AgNPs was also tested at ambient temperature. A UV-visible spectrophotometer (Optizen 2120, Daejeon), with an automatic rotary type-8 cell holder equipped with user-friendly Optizen view 4.1 software and quartz cuvettes with a path length of 1 cm, was used to analyze AgNP samples in the range of 300–800 nm. The SigmaPlot software (v 10.0, Systat Software, Inc. San Jose, USA) was used for the statistical analysis of spectral data.

Instrumentation

Fourier transform infrared spectroscopy (FT-IR) spectra of gallic acid, and AgNPs were acquired using potassium bromide pellets on a Nicolet™ iS50 (Thermo Fisher Scientific, Waltham, USA) in transmittance mode from 500 to 4000 cm^{-1}. High-resolution transmission electron microscopy (HR-TEM) was performed on a JEOL JSM-2100F, Tokyo, Japan to observe the shape, size distribution, and aggregation of the AgNPs. Dynamic laser scattering (DLS) and zeta potential measurements were performed after dilution of 2 mL of centrifuged AgNPs using 12 mL of nanopure water. Silver nanoparticle samples were then introduced into a folded capillary cell for zeta potential measurements using a temperature-controlled ELS-8000 (Otsuka, Japan) at 25 °C. For DLS, the AgNP sample was loaded into a quartz microcuvette, and three measurements were performed using a laser light scattering BI-9000AT spectrometer (Brookhaven Instruments, Holtsville, USA).

Selectivity of AgNPs

The selectivity of the developed analytical platform was investigated by observing the colorimetric changes and UV-visible spectral shifts. The AMG samples of kanamycin, amikacin, tobramycin, gentamycin, neomycin, and streptomycin were prepared by treating 0.5 mL of 1 nmol L^{-1} stock solutions with AgNPs (50 μL stock solution) dissolved in 0.45 mL of nanopure water. To employ the proposed colorimetric probe, as compared with AMGs, 100-fold molar excess stock solutions (100 nmol L^{-1}) of penicillin G, penicillin V, ampicillin, erythromycin, clarithromycin, azithromycin, tetracycline, chlortetracycline, oxytetracycline, metacycline, and doxycycline were used. The UV-visible spectra and colors recorded for the tested AMGs and other antibiotic samples helped characterize the optical and structural changes occurring in the AgNP dispersions.

Sensitivity of AgNPs to streptomycin

For the determination of streptomycin, a 50 μL AgNP probe solution was incubated with increasing concentrations of streptomycin from 50 to 650 pmol L^{-1} in 1 mL of water. Streptomycin-treated AgNP probe solutions were used to record UV-visible spectra for 20 min at ambient temperature. The LOD (A_{560}/A_{400} ratio, $n = 3$) of

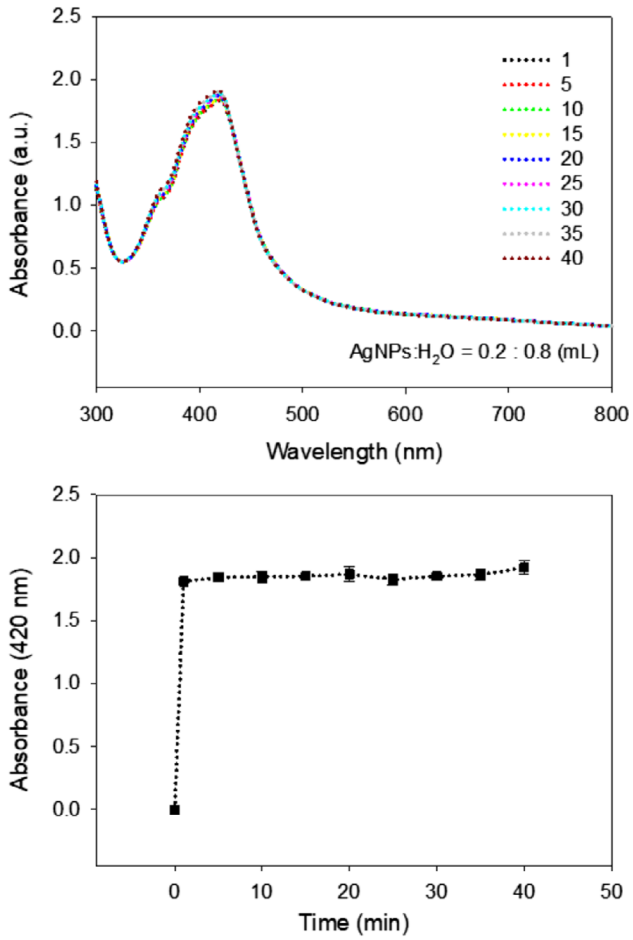


Figure 1. (a) Time optimization of synthesis reaction of AgNPs using gallic acid; (b) kinetic curve of AgNPs using gallic acid.

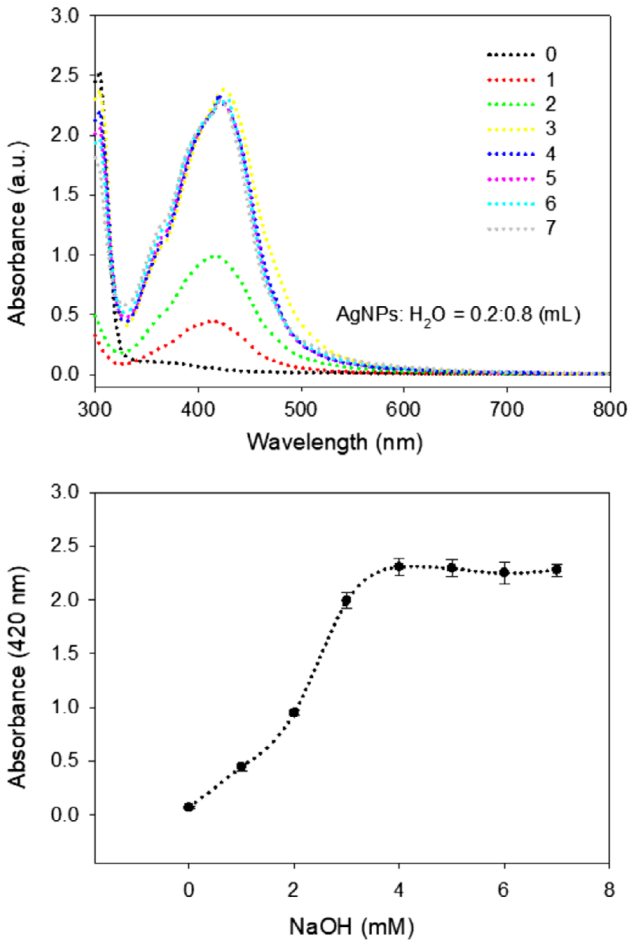


Figure 2. (a) Effect of NaOH concentration on UV-visible spectrum of AgNPs; (b) effect of NaOH concentration on kinetic curve of AgNPs.

the AgNP probe to streptomycin was calculated as $3\sigma/k$, where σ is the standard deviation and k is the slope of the linear calibration plot.

Real-time response of AgNPs toward streptomycin

The real-time absorbance response was evaluated as follows: 50 µL aliquots of the AgNP stock solution were suspended in 0.75, 0.55, or 0.35 mL of water, and initial absorbance intensities were measured at 400 and 560 nm. Subsequently, 0.2, 0.4, or 0.6 mL of streptomycin (1 nmol L^{-1}) was added to these solutions and absorbance intensities were again measured at 400 and 560 nm at 1 min intervals up to 31 min.

Effect of pH and ionic strength on streptomycin detection

The effects of pH and ionic strength on the performance of the AgNP probe were studied as follows: 50 µL of the AgNP stock solution was dispersed in 0.55 mL of nanopure water in UV-visible cuvettes at pH 2.2, 3.2, 4.3, 5.1, 6.2, 7.3, 8.3, or 9.3, and ionic strengths of 5, 10, 15, 20, 25, 30, or 35 mmol L^{-1}. The AgNP probe was incubated for 5 min, and absorbance intensities were measured at 560 nm ($n = 3$). Then, 400 µL of streptomycin (1 nmol L^{-1}) was added and mixed carefully. The AgNP probe was allowed to react for another 20 min at ambient temperature, and the absorbance intensities of the treated AgNP probe were again measured at 560 nm.

Analysis of streptomycin in spiked milk and serum samples

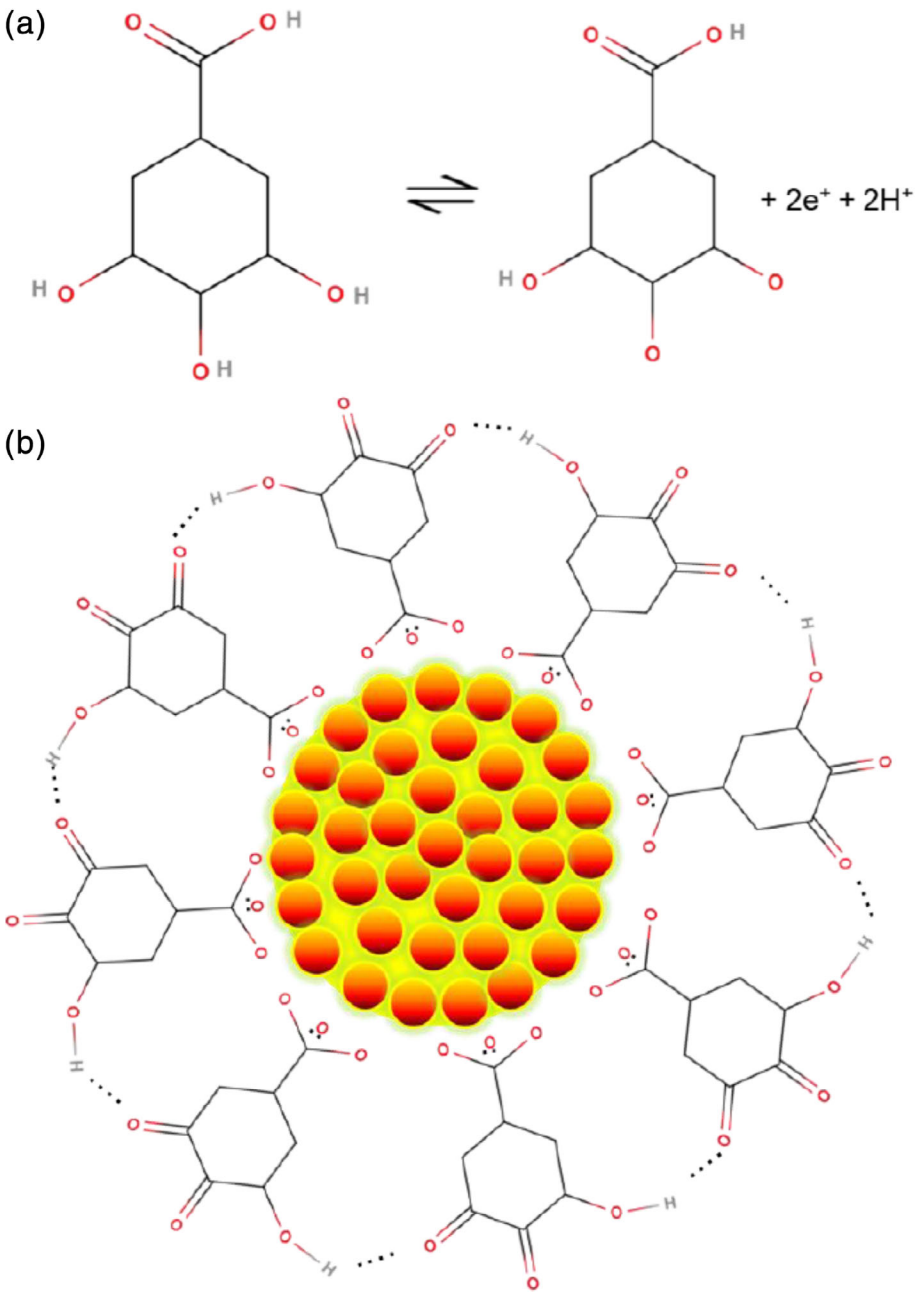
Fresh milk was bought from the local market, stored at 4 °C, and used as a real sample for the analysis of streptomycin-spiked residues. Real streptomycin samples were prepared by adding streptomycin to 1/50 dilutions of milk samples with nanopure water ($n = 3$), to obtain final streptomycin concentrations of 250 to 1750 pmol L^{-1}, which were analyzed in a process similar to the one undertaken for sensitivity analysis.

A fetal bovine serum sample (Sigma-Aldrich) was passed through syringe filters with a 0.2 µm membrane. Streptomycin-spiked samples were prepared after 1/50 dilutions of serum samples with nanopure water with final concentrations in the range of 100 to 1300 pmol L^{-1}, and were subsequently stored in a refrigerator (4 °C). These samples were analyzed in a process similar to the one undertaken for sensitivity analysis. The determination of streptomycin in milk and serum samples up to the picomolar level was confirmed by both colorimetric results and the spectrophotometric calibration plot. The LODs (A_{560}/A_{400} ratio, $n = 3$) of the AgNP probe to spiked streptomycin samples were calculated by the equation mentioned above.

RESULTS AND DISCUSSION

AgNP synthesis time

The redox potentials of the Ag^+ ion and gallic acid are 0.8 and 0.5 V, respectively, according to the thermodynamically favorable



Scheme 1. (a) Gallic acid structures evidences that the phenolic-OH oxidizes to its quinone forme, (b) AgNPs stabilizes through the interaction of the negative charge of carboxylic acid groups of gallic acid.

conversion of Ag^{+} to Ag^{0} at ambient temperature (24 °C).[14] In this study, 4 mL of gallic acid (12.5 µmol L^{-1}) was mixed with 5.45 mL nanopure water and 0.5 mL of $AgNO_3$ (20 mmol L^{-1}), and no color change was observed in the reaction mixture incubated for 5 min. Finally, 0.05 mL of NaOH (1 mol L^{-1}) was added under vigorous shaking to initiate the synthesis of monodispersed AgNPs. The appearance of a dark brown color within a few seconds at ambient temperature indicated a rapid growth of AgNPs: thus, the method abides by the principle of green chemistry termed 'design for energy efficiency'.[15]

The kinetics of AgNP production was first examined during a reduction reaction with gallic acid to determine the time required for AgNP synthesis. Ultraviolet-visible spectra of the AgNP solution were recorded at the first minute and then at intervals of 5 min after dilution of the AgNP solution with nanopure water (0.2:0.8 mL). The temporal evolution of the surface plasmon resonance (SPR) band in the reaction mixture was studied, and the same wavelength of 420 nm was observed (Fig. 1(a)). Based on these results, a fraction of 1 min is sufficient for the $AgNO_3$ reduction to reach completion, as demonstrated by the characteristic UV-visible spectra. The reduction of $AgNO_3$ to AgNPs was rapid and evident immediately after the early 1 min of synthesis, and the absorbance intensity recorded at 420 nm remained stable over the next 40 min (Fig. 1(b)). As the rate of nucleation was much higher, a large number of Ag nuclei formed immediately after the increase in the pH. The result represents the first instance in which this green method yielded monodisperse and highly stable AgNPs, with a noteworthy rate of nucleation and growth growth.

Finally, gallic acid efficiently reduced most of the Ag^+ ions to Ag^0 and stabilized AgNPs by the classical nucleation and growth route. This method also follows the green chemistry principle of the atom economy as it offers excellent conversion efficiency when converting the $AgNO_3$ precursor because it converts all of the atoms into the desired product.[16]

Effect of NaOH and gallic acid concentration

In brief, 4 mL of gallic acid (12.5 µmol L^{-1}) was first added to 0.5 mL of AgNO3 (20 mmol L^{-1}), followed by the addition of dilute NaOH in a range from 1 to 7 mmol L^{-1}. Ultraviolet-visible spectra revealed the appearance of characteristic SPR bands without any adverse effects on any of the tested NaOH concentrations (Fig. 2(a)). These results suggest that two types of functional groups, carboxylic acid and hydroxyl, present on the gallic acid molecule, act synergistically in the reduction of $AgNO_3$ at an alkaline pH. The pKa of gallic acid (OH, ~9.8 and – COOH, ~4.4)[14] suggests that it normally exists in the ionized form at neutral and near-alkaline conditions, which can enhance the electrostatic interaction of gallic acid with the AgNPs' surface. For this method, the initial concentration of 3 mmol L^{-1} NaOH showed the highest absorbance intensity and yield of AgNPs. The AgNPs' productivity remained identical with further increases in the NaOH concentration (Fig. 2(b)). In this method, the dihydroxyl groups of gallic acid engage in a two-electron oxidation reaction to result in a subordinate quinone form (Scheme 1(a)). Thus, the AgNPs as synthesized were completely stabilized through the interface of negative carboxylic groups. To the best of our knowledge, gallic acid molecules undergo an electron oxidation process (Scheme 1(a)), where the initial concentration of NaOH is vital for the improved synthesis of the desired AgNPs.[17]

The results shown in Fig. 3(a) demonstrated that the UV-visible spectra of the AgNPs that were obtained remain intact with the increasing concentrations of gallic acid. Typically, the synthesis of AgNPs is not efficient at low concentrations of gallic acid (~0.312 mmol L^{-1}, Fig. 3(a), red line), but the SPR band of AgNPs did achieve the maximum height at slightly higher concentrations (~0.625 mmol L^{-1}). Higher concentrations of gallic acid in the tested range of 1.25 to 7.5 mmol L^{-1} resulted in AgNP reaction mixtures with consistent color, SPR bands, and absorbance intensities at 420 nm (Fig. 3(a), (b)). Our results indicate that the initial concentration of gallic acid does affect the AgNP yield (Fig. 3(b)). Furthermore, green synthesis, achieved by gallic acid-mediated reduction of $AgNO_3$ is interesting because it can act simultaneously as a rapid reducing and stabilizing agent.[18]

Characterization of AgNPs

Zeta potential measurements of freshly prepared and centrifuged AgNP samples after several weeks were – 26.8 and – 19.6, respectively. Both the AgNPs suspensions could satisfactorily resist aggregation, and showed excellent aqueous dispersion stability. Ultraviolet-visible spectroscopy was also used to characterize the optical properties and stability of colloidal dispersions of AgNPs. As shown in Fig. 4(a), the SPR band of AgNPs appeared at 420 nm for before the centrifugation (BC) sample; it also showed sensitivity that was too low for the picomolar-level detection of AMG antibiotics. The AgNP probe solution obtained after centrifugation (AC) at 14 600 g for 15 min was supposed to exhibit desirable properties in terms of color intensity and blue-shift towards a lower wavelength at 400 nm. However, the optical

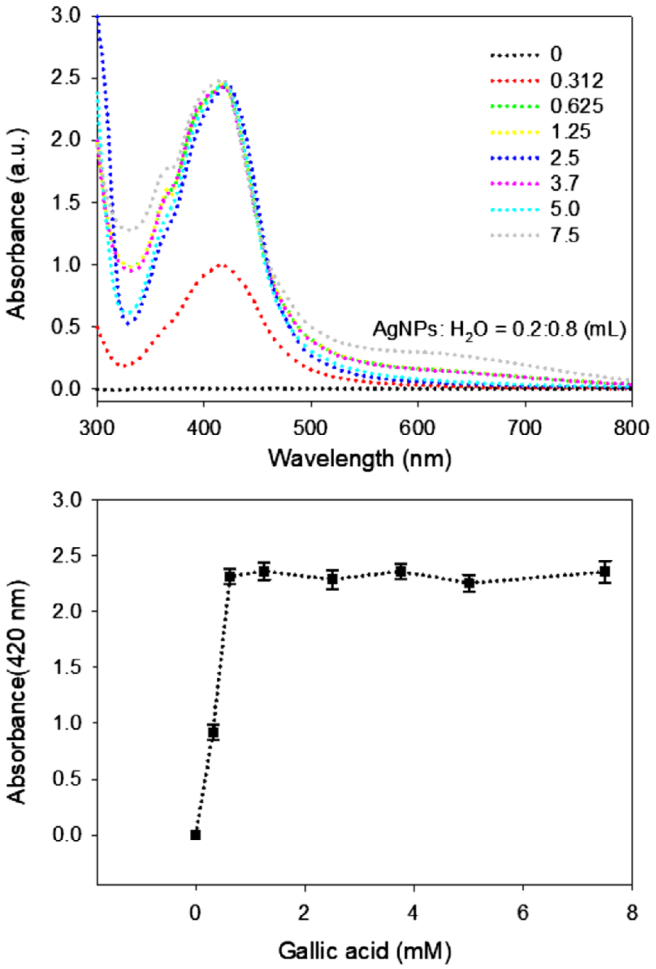


Figure 3. Effect of gallic acid concentration on UV-visible spectrum of AgNPs; (b) effect of gallic acid concentration on kinetic curve of AgNPs.

properties of AgNPs:AC provided sustained colloidal stability and high sensitivity towards AMGs.

Characterization of AgNPs by DLS, HR-TEM, and FT-IR provided essential information about the functional groups present on the AgNPs. Gallic acid facilitated the formation of monodisperse AgNPs with a narrow size distribution that could be observed directly by the HR-TEM image (Fig. 4(b)), with a mean diameter of approximately 17.5 nm. The average hydrodynamic diameter of the AgNPs was 20.4 nm, with a size distribution of 15 – 25 nm, in agreement with HR-TEM results (Fig. 4(c)).

Figure 4(d) shows the FT-IR spectra of pure gallic acid and AgNPs. These spectra provided essential information about the surface functional groups on AgNPs. The broad FT-IR band at 3230 – 3360 cm^{-1} could be allocated to the stretching vibration of carboxylic groups (– OH). The position of the peak above shifted to a slightly higher wave number after the reduction of Ag^+ with gallic acid. This result indicates that phenolic -OH was oxidized to its quinone form, as illustrated in Scheme 1(b), where it produces an electron to reduce Ag^+ and finally the carboxylic group bonds and stabilizes the resulting AgNP surfaces. The peak at 1215 cm^{-1}, owing to the C—O stretching vibrations, disappeared after the interaction of gallic acid with Ag^+. Vibrational peaks from the fingerprint region also appeared at 1616, 1541, and 1353 cm^{-1} as seen in Fig. 4(d), owing to the interaction of aromatic rings of

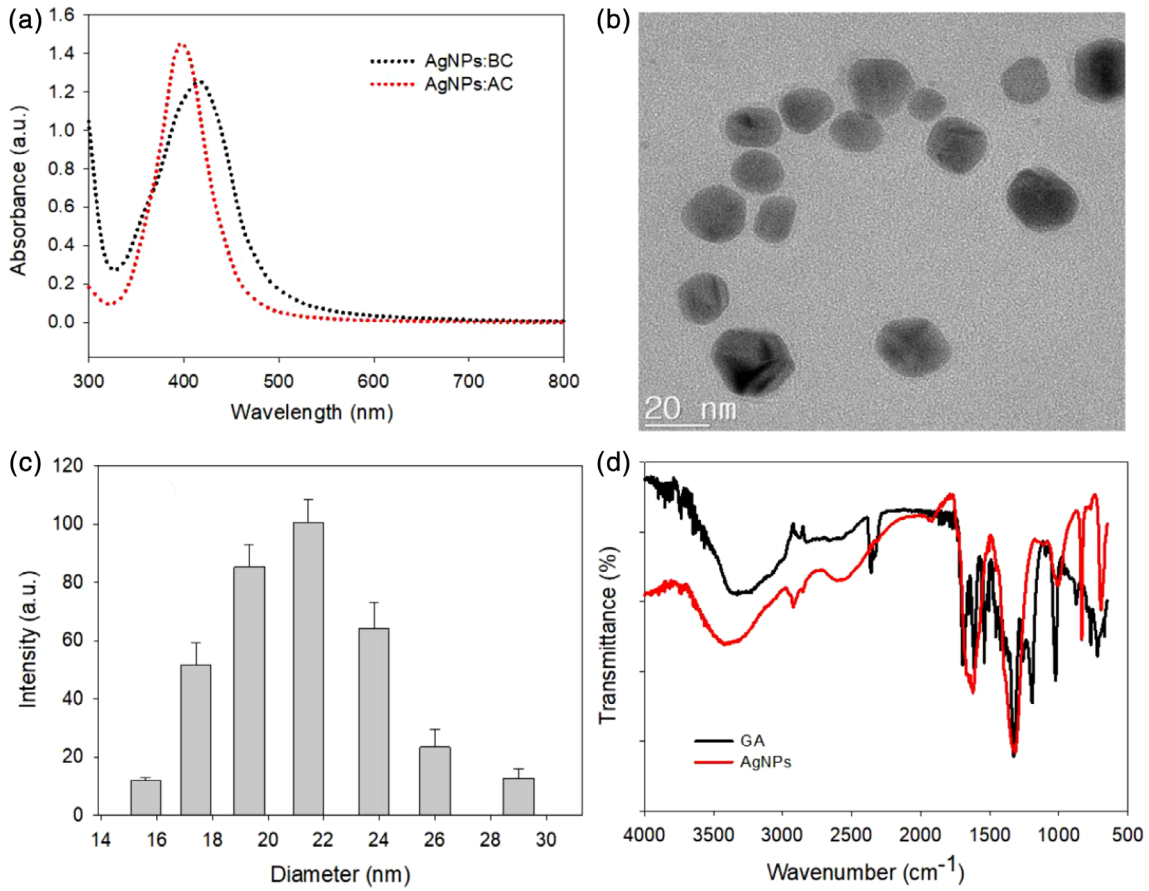


Figure 4. (a) Effect of centrifugation on UV-visible spectrum of AgNP probe; (b) HR-TEM image of AgNPs; (c) dynamic light scattering of AgNP probe; (d) FT-IR spectra of gallic acid (black line) and AgNP probe (red line).

gallic acid with Ag^+. These observations agree with those of Yoosaf *et al.*,[19] who reported that a peak at 3422 shows that the phenolic group around the AgNPs engages in intermolecular hydrogen bonding (as illustrated in Scheme 2).

Visual detection of aminoglycoside antibiotics

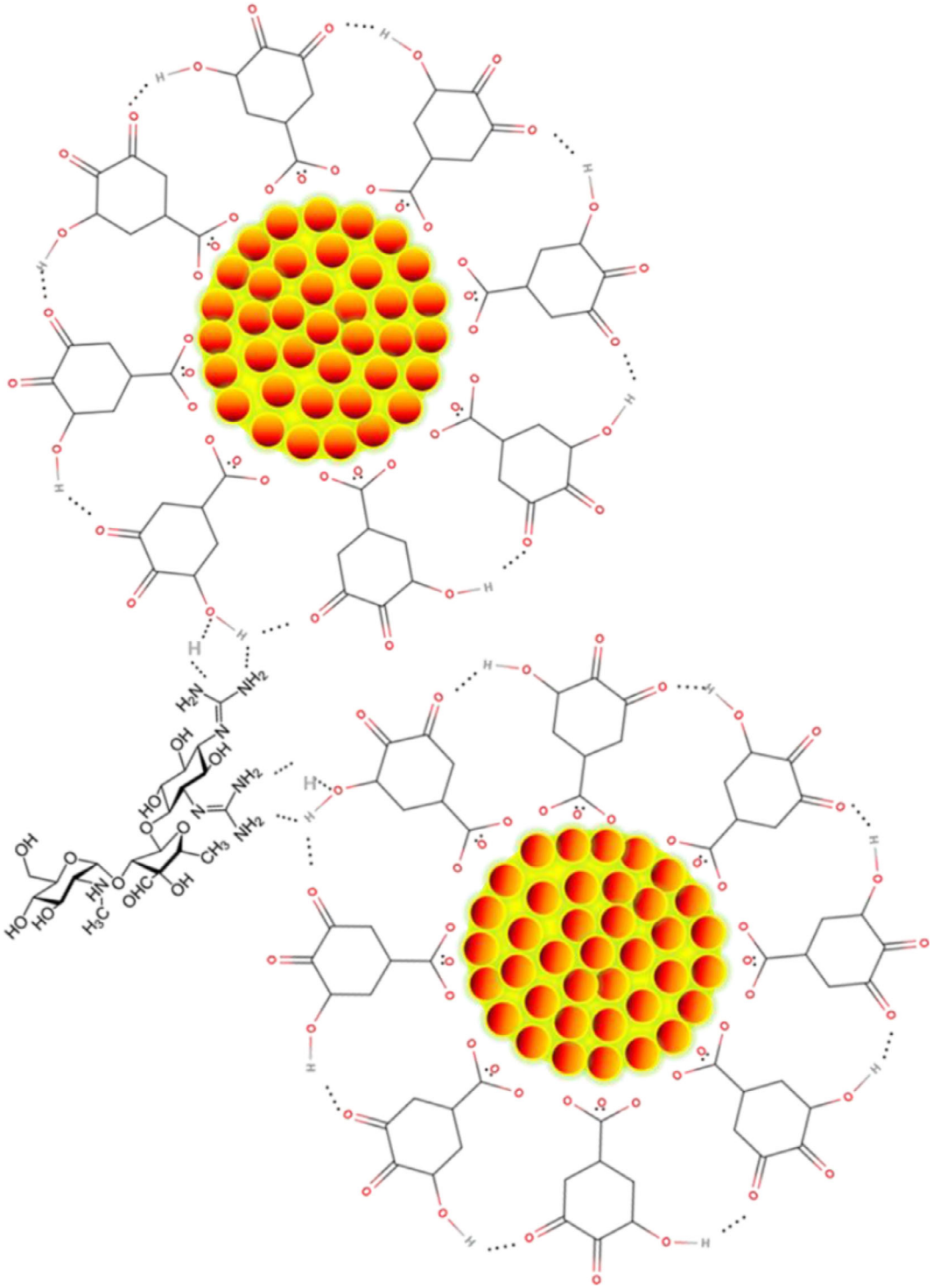
The effects of a range of other types of antibiotics on the optical properties of AgNPs were investigated at a 100-times higher concentration than that of common AMGs (Fig. 5). No sign of significant broadening of the SPR band was observed as compared to the red shift for AMG antibiotics observed under identical conditions. The larger wavelength plasmon band shift for all tested AMG antibiotics typically originates from the coupling of the plasmon resonance with that of neighboring AgNPs (Fig. 5). This confirms that plasmon coupling interaction specifically occurs with AMGs, resulting in an identical bathochromic shift to the original SPR band (Fig. 5). This study thus confirms that gallic acid-coated AgNPs are highly specific toward AMG antibiotics. Previously, different techniques including localized SPR and surface-enhanced Raman spectroscopy for the detection of AMG antibiotics have been reported with an in-depth study of tobramycin using citrate-capped gold NPs.[20]

Determination of streptomycin concentration in water

Considering the extensive use of streptomycin in agriculture and animal husbandry, an in-depth study on the detection of streptomycin was conducted, taking it to be a model AMG antibiotic. As shown in Fig. 6(a), a linear decrease was noted in the absorbance intensity at 400 nm with the addition of increasing concentrations of streptomycin. In contrast, a linear increase in the absorbance intensity was observed at 560 nm with increasing concentration of streptomycin. Thus, the intensity of the red-shifted band was sensitively dependent on the concentration of streptomycin, and the plateauing state was reached at 650 pmol L^{-1}. A color change from yellow to orange or red was observed with a further increase in the intensity of the red-shifted band (Fig. 6(a)) when the amount of streptomycin was increased from 200 to 650 pmol L^{-1}. The dependence of the ratiometric results (A_{560}/A_{400}) on the concentration of streptomycin was expressed in a calibration plot over a range of 50 to 650 pmol L^{-1} of streptomycin, which showed a coefficient of determination (R^2) of 0.987 (Fig. 6(b)). To the best of our knowledge, this is currently the most sensitive method for colorimetric detection and quantification of streptomycin in water, with an LOD of about 36 pmol L^{-1}. A comparison of the sensitivity of streptomycin detection using this method with that of existing methods is summarized in Table 1. Detection of streptomycin visually was possible at a concentration of about 200 pmol L^{-1} (Fig. 6(a)), which is far lower than the MRL set for streptomycin by the European Union, China, and the World Health Organization.[21]

Zeta potential measurement

This report further exploits the polycationic nature of streptomycin antibiotics using negatively charged AgNPs. The surface charge of the AgNPs can be controlled by coating the NP surface by



Scheme 2. Gallic acid-aminoglycoside coordination complex drives the aggregation of AgNPs.

gallic ions, providing a strong negative charge. A stable dispersion of AgNPs was achieved by the repulsive electrostatic interactions at the NP interface, causing AgNPs to remain stable in aqueous media (Fig. 7(a)). The zeta potential of the solution gradually rose because, as the positive charge arising from interactions with streptomycin increased, it partially neutralized the negative charge of the gallic acid-functionalized AgNPs. The zeta potentials linearly increased from −19.6 to −1.57 mV upon the addition of 200 to 600 pmol L^{-1} of streptomycin, as seen in Fig. 7(a), assuming the net charge reached zero, and carboxylic acid and guanidinium groups remain deprotonated and protonated, respectively. Attractive electrostatic interactions led to the aggregation of AgNPs dependent on the streptomycin concentration, which was further confirmed by HR-TEM imaging (Fig. 7(b)). In the presence of AgNPs, the target molecules first bound with gallic acid to form the streptomycin-AgNP complex and further aggregates of AgNPs.[9]

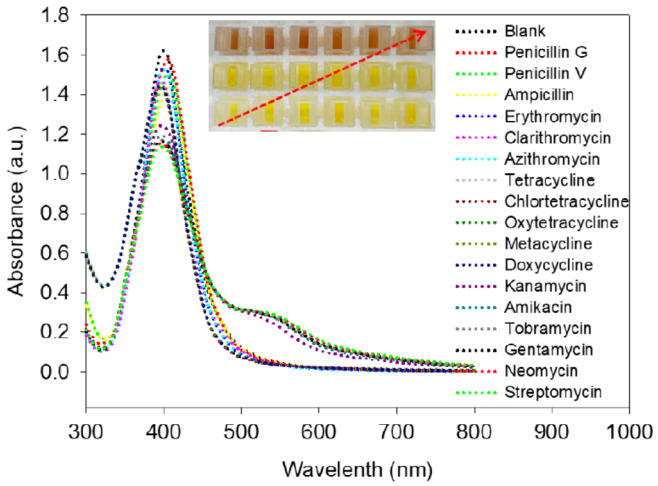


Figure 5. (a) Selectivity of of AgNP probe over AMGs (500 pmol L^{-1}) and other antibiotics (50 nmol L^{-1}) (inset, photo of AgNP probe with different antibiotics).

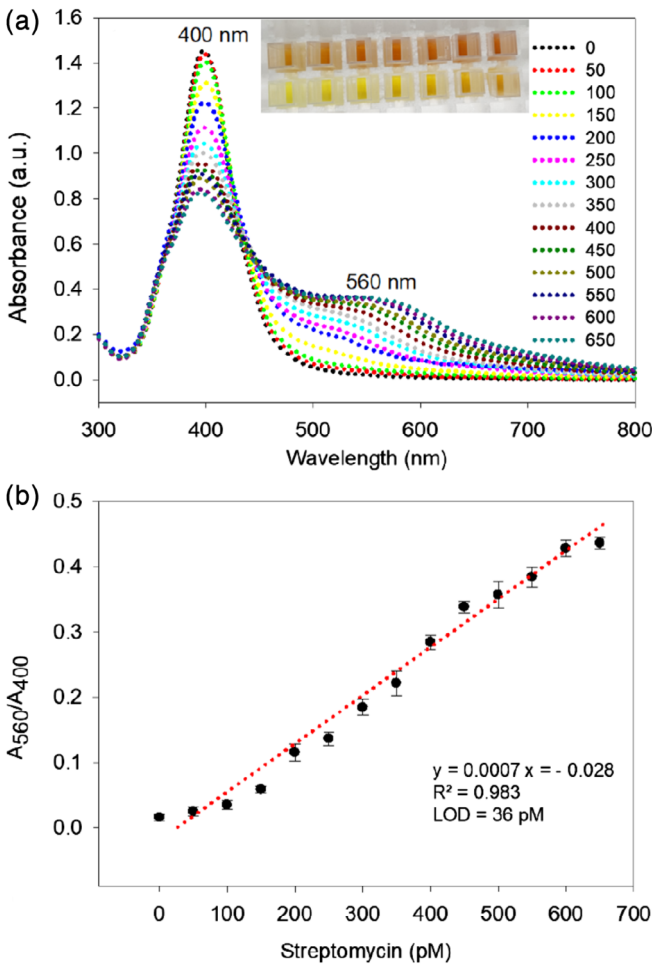


Figure 6. (a) Ultraviolet-visible spectra of AgNP probe with increasing concentrations streptomycin in water; (b) calibration plot of absorbance ratio (A_{560}/A_{400} nm) (inset, photo of AgNP probe after streptomycin bonding in water).

Real-time response

Figure 8(a),(b) present the real-time absorbance response of AgNPs at three different concentrations of streptomycin. Time-dependent changes in the absorbance intensity (Fig. 8(a),(b)) were directly investigated by adding 200, 300, or 400 pmol L^{-1} of streptomycin to the solution of AgNPs. Silver nanoparticle aggregates were fully assembled within 20 to 30 min irrespective of the streptomycin concentration, suggesting 20 min as an appropriate time for streptomycin detection. Notably, the absorbance intensity at 400 nm gradually increased to the maximum, and complete stability of the nanoprobe was achieved within 20–30 min (Fig. 8(a)). The streptomycin concentration-dependent temporal increase in the absorbance of the red-shifted wavelength at 560 nm (Fig. 8(b)) suggests the possibility of precise tuning of the fractal growth of AgNP aggregates.[22]

Effect of pH and ionic strength

The pH of the AgNP probe solution has a significant effect on the detection of the analyte. The streptomycin detection ability of AgNPs at a variety of pH from 2.2 to 9.3 was investigated for a 400 pmol L^{-1} concentration of streptomycin. Figure 8(c) represents the dependence of the absorbance intensity (560 nm) on the pH in the absence and presence of streptomycin. Results show that the AgNPs were stable at neutral and alkaline pH, with the maximum response to streptomycin under near-acidic and basic conditions (Fig. 8(c)). Silver nanoparticles exhibited the strongest colorimetric signal for streptomycin in the range of pH 6.2 to 8.3. At a pH higher than 9.3, the aggregation of AgNPs was likely suppressed due to the deprotonation of streptomycin. In addition to this, an increase in the dissociation of hydroxyl groups of gallic acid present on the AgNP surfaces occurred, leading to an increase in the overall charge of the solution and added stability. A higher number of protonated amino and deprotonated hydroxyl groups of streptomycin and gallic acid led to the greater aggregation of AgNPs.[20] A similar observation been reported for gold NPs and citrate ions used in the detection of neomycin, another AMG antibiotic.[22]

Ionic strength is another important parameter for improving the detection of target analytes, and the dependence of AgNPs absorbance response in the presence of diluted NaCl (5 to 35 mmol L^{-1}) was investigated. Results are presented in Fig. 8(d). Absorbance intensity (560 nm) changes after addition of streptomycin to the increasing concentrations of NaCl (5 to 35 mmol L^{-1}) was found to be a dose-dependent manner, indicating an increase in detection sensitivity correlated with ionic strength (Fig. 8(d)). Thus, Na^+ cations can be key to enhancing the sensitivity of AgNP

Table 1. Determination of streptomycin in spiked real samples by different methods

Method	Sample	Range (nmol L^{-1})	LOD (nmol L^{-1})	Reference
Immunobiosensor	Milk	31.8–3180	13.03	26
Fluorescence	Serum	30–2030	47.6	27
Competitive ELISA	Milk	0.31–3180	6.36	28
Colorimetric gold NPs	Buffer	100–500	86	9
Electrochemical	Milk	0.15–318	1.59	1
Colorimetric gold NPs	Honey	200–1200	200	29
Fluorescent aptasensor	Buffer	50–1060	54.5	30
Colorimetric silver NPs	Milk	0.25–1.75	0.179	This method
Colorimetric silver NPs	Serum	0.1–1.0	0.138	This method
Colorimetric silver NPs	Water	0.05–0.750	0.036	This method

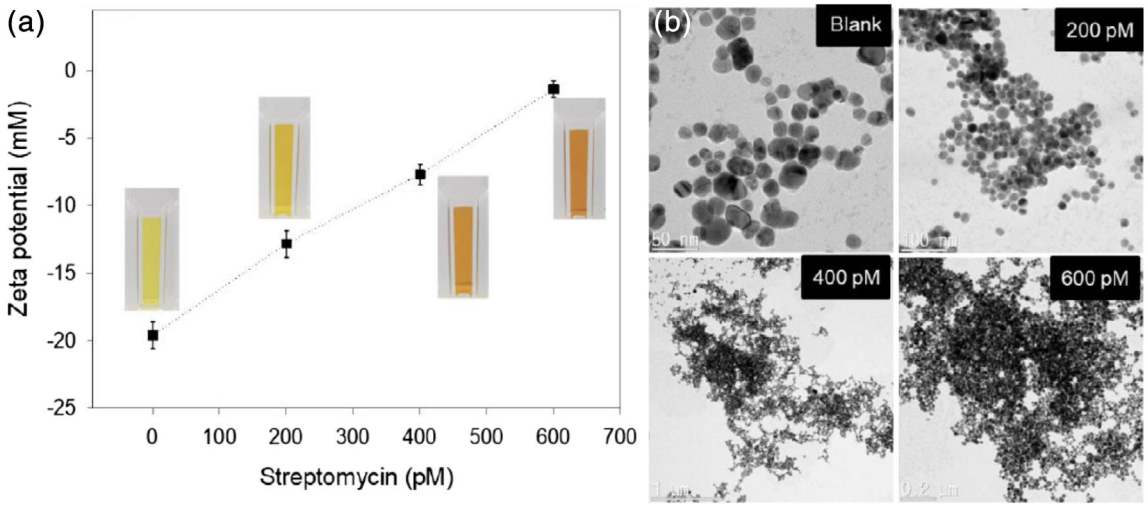


Figure 7. (a) Zeta potential of AgNP probe at different streptomycin concentrations (inset, photo of color changes of AgNP probe at different concentrations of streptomycin); (b) HR-TEM images of AgNP probe and their aggregates at different concentrations of streptomycin (0, 200, 400, and 600 pmol L^{-1}).

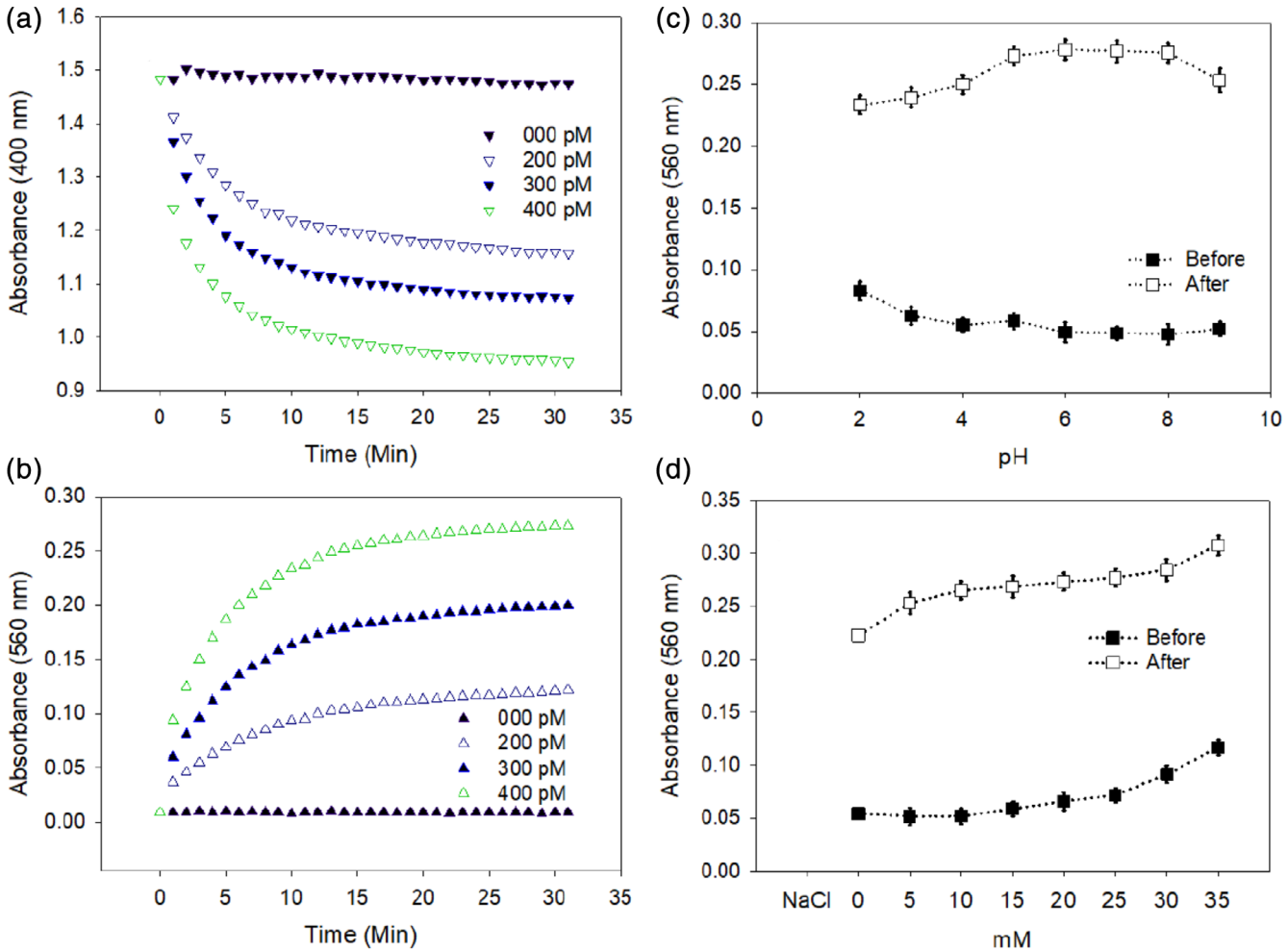


Figure 8. (a) Time course of the absorbance response of AgNP probe recorded at 400 nm in the presence of streptomycin (200, 300, and 400 pmol L^{-1}); (b) time course of the absorbance response of AgNP probe recorded at 560 nm in the presence of streptomycin (200, 300, and 400 pmol L^{-1}); (c) absorbance intensity of AgNP probe recorded at 560 nm in the presence and absence of streptomycin at different pH levels; (d) effect of ionic strength on absorbance intensity of AgNP probe recorded at 560 nm in the presence and absence of streptomycin.

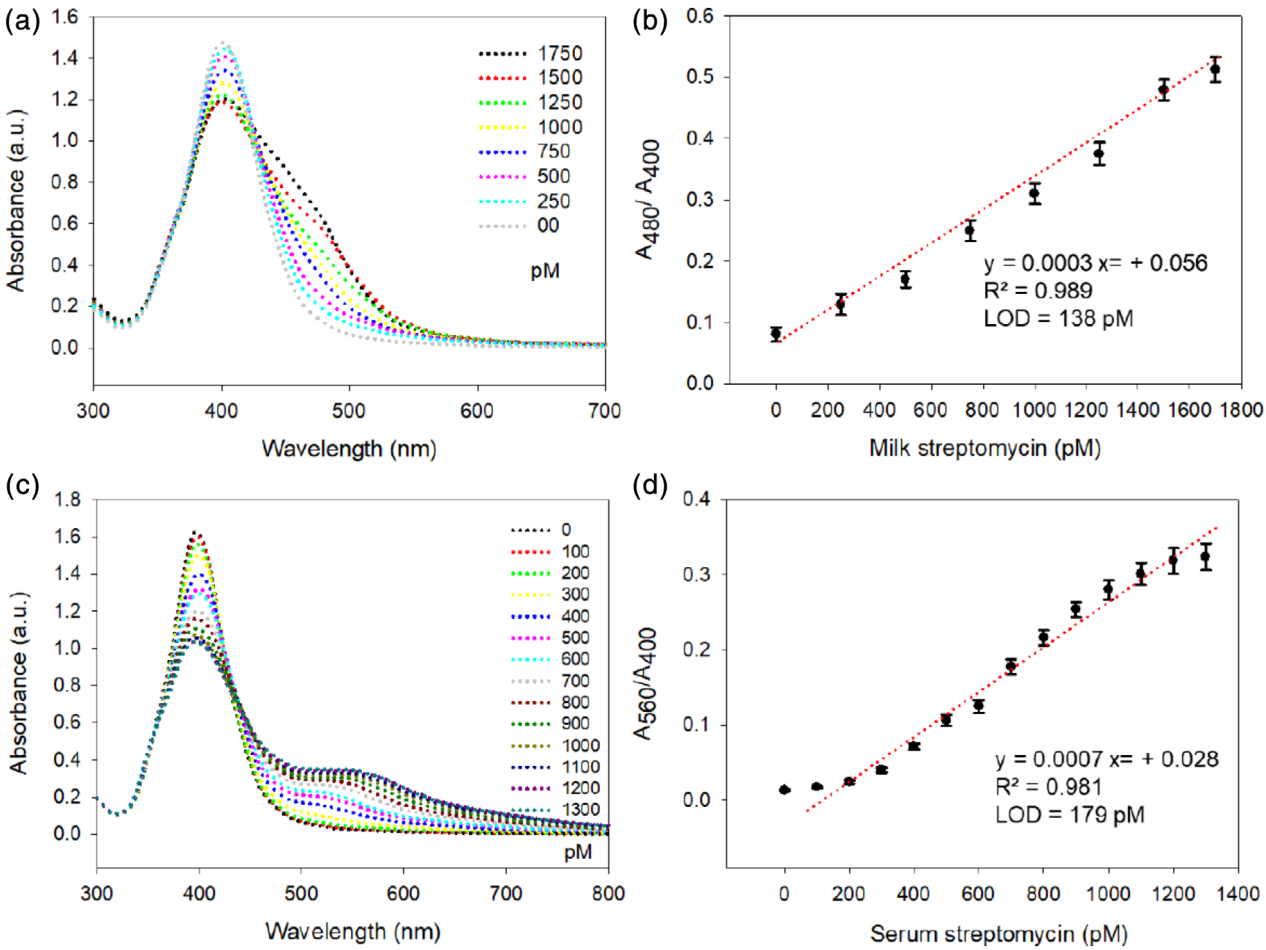


Figure 9. (a) Ultraviolet-visible spectra of AgNP probe after mixing with different streptomycin concentrations in milk; (b) linear calibration plot of absorbance ratio (A_{480}/A_{400} nm) against different streptomycin concentrations in milk; (c) ultraviolet-visible spectra of AgNP probe with different streptomycin concentrations in serum; (d) linear calibration plot of absorbance ratio (A_{560}/A_{400} nm) against different streptomycin concentrations in serum.

probes as they penetrate the layer of negatively charged AgNPs and may decrease its zeta potential charge. Ionic strength is recognized to cause additional aggregation of the AgNPs, therefore we surmise that Na^+ cations facilitate the aggregation of AgNPs via an electrostatic screening effect.[9] This effect leads to an increase in electrostatic attraction and sensitivity of the detection system designed for target analyte streptomycin by resulting in intensified AgNP aggregation.

Real samples analysis

We tested the practical applicability of the developed AgNP probe by using it to detect streptomycin residues in commercially obtained milk samples. Milk samples were first diluted to 1:50 with nanopure water and used to measure picomolar levels of streptomycin in milk. A calibration plot between the ratio of absorbance (A_{480}/A_{400}) and the streptomycin spiked in milk was obtained in the range from 250 to 1750 pmol L^{-1} with an R^2 value of about 0.987 and a LOD of about 179 pmol L^{-1} (Fig. 9(a),(b)). A number of laboratory-based methods and commercial test kits use microbial inhibition assays for the detection of antibiotics. However, most of those methods are either unable to detect AMGs or their LODs are much higher than that of the MRL values.[23]

We also evaluated the use of the AgNP probe in the practical detection of streptomycin residues in serum samples. Linear relationships between the ratiometric (A_{560}/A_{400}) response and streptomycin concentrations in serum were found to be suitable when calibration plots were obtained for the range from 100 to 1300 pmol L^{-1}, with an R^2 value of about 0.987. The ratiometric response correlated directly with the concentration of streptomycin in the serum samples, with a LOD of about 138 nmol L^{-1} (Fig. 9(c),(d)). The LODs determined were much lower than those for existing methods (Table 1) as well as documented toxicity levels (30–40 µg mL^{-1} in blood and 200 ng mL^{-1} in milk).[24]

A critical factor determining the suitability of the probe is the sensitivity of the designed ligand; here, we confirmed the potential for the easy detection of AMGs down to the lowest MRL value. The results also demonstrate that an AgNP probe is appropriate for the detection of streptomycin in real samples and the monitoring of milk and serum samples for the residual presence of streptomycin. The selectivity data suggested important implications in the context of food safety inspection, as this method could be applied to the detection of other AMG antibiotics by preparing appropriate calibration plots. Although a number of probes specific to a particular AMG have been reported before,[25] a common probe selective for broad specificity has been identified in this work.

CONCLUSIONS

A colorimetric nanoprobe based on gallic acid-functionalized AgNPs was developed for the detection of AMG antibiotics. The

AMG-induced changes in color from yellow to orange or red can be readily visualized, and showed not only high selectivity but also sensitivity. The AgNPs showed high sensitivity upon saturation with streptomycin, suggesting that the developed ratiometric absorbance probe is suitable for the analysis of water, serum, and milk samples with LODs of about 36, 138, and 179 pmol L^{-1}, respectively. The AgNP probe readily bonds with streptomycin and forms aggregates, which result in a response in terms of a substantial shift in the colorimetric peak at around 560 nm. The new type of nanoprobe using an aggregation-induced absorbance response can provide a practical method for the detection of AMGs in aqueous solutions. The developed AgNP probe was found to be applicable for the colorimetric detection of AMGs down to picomolar level. The probe can be prepared quickly due to the ease of AgNPs functionalization and the overall speed of AMGs detection (∼1 min), without the involvement of any sophisticated techniques or expensive reagents.

ACKNOWLEDGEMENTS

This research was supported by Dongguk University-Seoul research fund 2018-2020. The authors extend their appreciation to the researchers supporting project number (RSP-2019/56) King Saud University, Riyadh, Saudi Arabia.

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