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One-step functionalization of gold and silver nanoparticles by ampicillin

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ABSTRACT

This report describes a one-step functionalization of gold and silver nanoparticles with ampicillin in which ampicillin is also used as a reducing agent to convert Au^{3+} and Ag^{+} to gold and silver nanoparticles, respectively. This process eliminates the use of other chemical reducing agents to provide a completely green synthetic route. The nanostructure, morphology, crystallinity, and reaction yield of the nanoparticles were investigated by spectroscopic and microscopic techniques. Spherical and amorphous nanoparticles were synthesized with diameters of 10–33 nm. Remarkably, the newly-prepared NPs show excellent antibacterial activities against *Streptococcus pyogenes* (minimum inhibitory concentration 0.14–1.09 μg/mL). This system enables the straightforward functionalization of gold and silver nanoparticles with ampicillin using a simple, one-step process.

1. Introduction

The advent of multidrug resistant (MDR) bacteria has necessitated the development of novel categories of antibiotics that effectively block or subvert bacterial growth. Metallic nanoparticles are an effective platform for antibacterial nanotechnology. In particular, silver nanoparticles (AgNPs) have shown promise as bactericidal agents for MDR bacteria [1–4]. Gold nanoparticles (AuNPs) and AuNPs functionalized/mixed with antibiotics are also effective antibacterial agents and enhance the antibacterial activity of antibiotics [5–11]. Brown et al. have reported that the functionalization of AuNPs and AgNPs with ampicillin destroys MDR bacteria [12]. They prepared citrate-stabilized NPs using $NaBH_4$ as a reducing agent and then mixed the NPs with ampicillin to generate ampicillin-functionalized NPs [12]. Kora et al. observed an enhancement of the antibacterial activity of AgNPs when they were combined with streptomycin, ampicillin, or tetracycline [13]. The combination of ampicillin with biogenic AgNPs synthesized by the fungus *Trichoderma viride* yielded a synergistic effect [14]. In these previous studies [12–14], the authors performed the experiments in two separate steps: preparation of the NPs and functionalization of the NPs with antibiotics.

In the present work, we develop a one-step synthesis of ampicillin–functionalized AuNPs and AgNPs (referred to hereafter as ampicillin–AuNPs and ampicillin–AgNPs). In this one-step synthesis, ampicillin functions as the reducing agent, thereby enabling a greener process by avoiding the use of other chemical reducing or capping agents. The ampicillin–functionalized NPs were characterized by inductively coupled plasma mass spectrometry (ICP-MS), Fourier transform infrared spectroscopy (FTIR), UV–visible spectrophotometry, high-resolution X-ray diffraction (HR-XRD), high-resolution transmission electron microscopy (HR-TEM), atomic force microscopy (AFM), and field emission scanning electron microscopy (FE-SEM).

2. Experimental

Materials and instruments: Ampicillin trihydrate, silver nitrate, and hydrochloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade. AFM images were acquired with a Dimension® Icon® (Bruker Nano, Santa Barbara, CA, USA) operated in tapping mode. A Shimadzu UV-1800 was used to acquire UV–visible spectra (Shimadzu Corporation, Kyoto, Japan). A JEM-3010 (JEOL, Tokyo, Japan) at 300 kV was

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used to obtain HR-TEM images. A JSM-7100F SEM with an accelerating voltage of 15 kV was used to collect FE-SEM images (JEOL, Tokyo, Japan). An ELAN 6100 (Perkin-Elmer SCIEX, Waltham, MA, USA) was used for ICP-MS. To obtain the supernatant containing unreacted Au^{3+} or Ag^{+} for ICP-MS analysis, the NPs were subjected to ultracentrifugation in an Eppendorf centrifuge 5424R (Eppendorf AG, Hamberg, Germany) at 21,000 g for 30 min at 21 °C for AuNPs and 21,130 g for 45 min at 18 °C for AgNPs. A high-resolution X-ray diffractometer (Bruker D8 Discover, Bruker, Germany) with a CuKα radiation source (λ=0.1541 nm) was used to obtain HR-XRD data. FT-IR spectra were recorded on a Varian 640-IR using the KBr pellet method (Agilent Technologies, Santa Clara, USA).

Functionalization of AuNPs and AgNPs by ampicillin: Ampicillin–AuNPs were prepared by mixing a $HAuCl_4 \cdot 3H_2O$ solution (1 mM in deionized water, 150 μL) with ampicillin (1 mM in deionized water, 50 μL). To prepare ampicillin–AgNPs, a silver nitrate solution (1 mM in deionized water, 300 μL) was mixed with ampicillin (0.5 mM in deionized water, 100 μL). The final volume of each mixture was adjusted to 1 mL with deionized water. The reaction was conducted in an 80 °C oven for 15 h (ampicillin–AuNPs) or 24 h (ampicillin–AgNPs). For antibacterial activities, twenty strains of Gram-positive and Gram-negative bacteria were utilized. Minimum inhibitory concentration (MIC) values were measured according to our previous report [15].

3. Results and discussion

Functionalization and yield of AuNPs and AgNPs: Surface plasmon resonance bands emerged at 527 nm with a pink color for the ampicillin–AuNPs and at 390 nm with a yellow-green color for the ampicillin–AgNPs (Fig. 1A). The reaction yield was 96.7% and 94.1% for the ampicillin–AuNPs and ampicillin–AgNPs, respectively, as determined by ICP-MS.

FT-IR spectra: As depicted in Fig. 1B, the FT-IR spectra changed greatly upon the synthesis of the NPs. The intense band for ampicillin at 1774 cm^{-1} disappeared completely, suggesting that the C=O functional group on ampicillin contributed to the synthesis of the NPs. The stretching vibration of the primary and secondary amines was observed at 3509 cm^{-1} and 3446 cm^{-1} for the ampicillin standard. These two bands merged, broadened, and shifted to 3418 cm^{-1} and 3436 cm^{-1} during the synthesis of the ampicillin–AuNPs and ampicillin–AgNPs, respectively, indicating that the amine functional group was involved in the synthesis and/or was complexed to the surface of the NPs.

HR-XRD analyses: As shown in Fig. 1C, the diffraction peaks of the ampicillin–AuNPs at 38.3°, 44.3°, 64.8°, and 77.6° represent the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of the Au crystal. The (1 1 1) plane of the ampicillin–AgNPs was also observed at 38.1°, confirming the crystallinity of the AgNPs.

HR-TEM images and size histogram: HR-TEM revealed spherical ampicillin–AuNPs with an average diameter of 18.71 ± 2.90 nm among 51 discrete NPs (Fig. 2). Large and small ampicillin–AgNPs with diameters of 33.96 ± 4.96 nm from 20 discrete NPs and 10.82 ± 3.06 nm from 36 discrete NPs, respectively, were observed (Fig. 3). Both spherical and amorphous AgNPs were observed.

AFM and FE-SEM images: As shown in Fig. 4A–C, AFM revealed spherical ampicillin–AuNPs. Spherical and amorphous ampicillin–AgNPs were clearly visualized in the AFM images (Fig. 4D–F). The AFM images correlated well with the HR-TEM images. The FE-SEM images also provided information about the shapes of the NPs (Fig. 5).

Antibacterial activities: Interestingly, the antibacterial activity of NPs against Gram-positive bacteria was higher than that of Gram-negative bacteria (Table 1). In addition, ampicillin–AgNPs

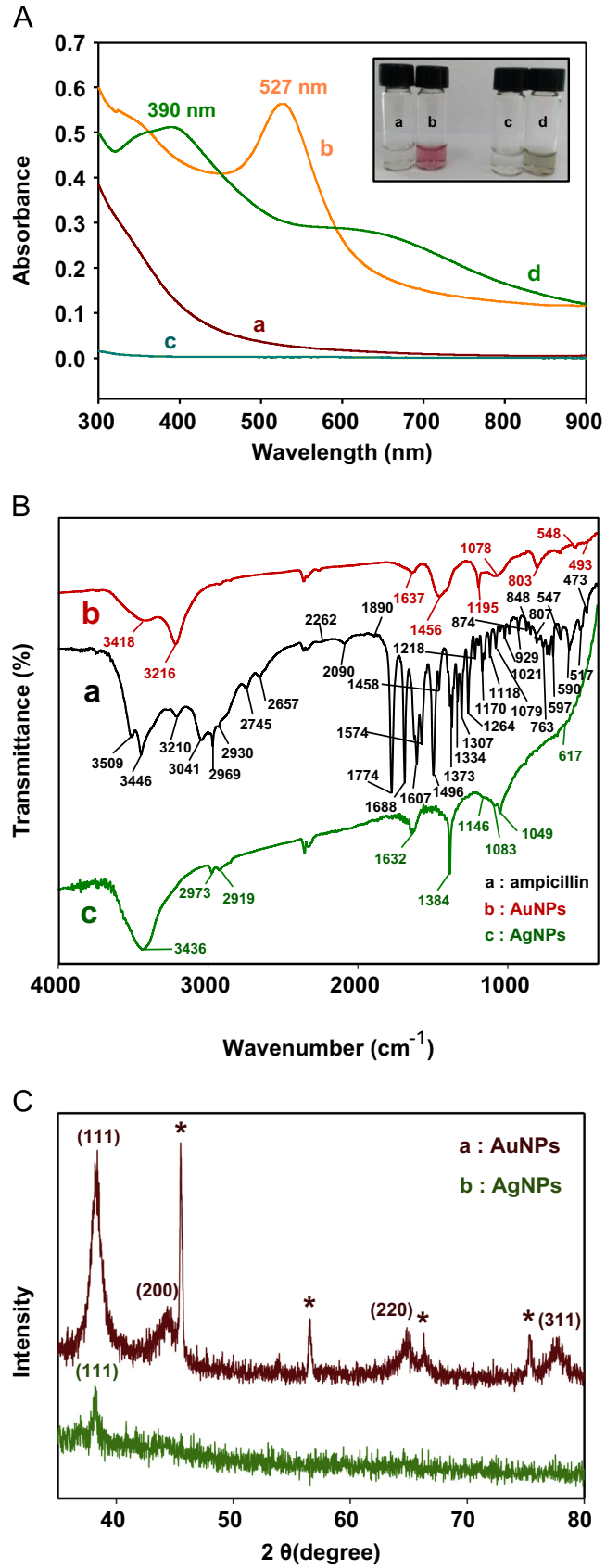

Fig. 1. (A) UV–visible spectra: (a) ampicillin–AuNPs before oven incubation; (b) ampicillin–AuNPs after oven incubation; (c) ampicillin–AgNPs before oven incubation; (d) ampicillin–AgNPs after oven incubation. (B) FT-IR analyses: (a) ampicillin; (b) ampicillin–AuNPs; (c) ampicillin–AgNPs. (C) HR-XRD analyses: (a) ampicillin–AuNPs; (b) ampicillin–AgNPs. The asterisk (*) indicates the presence of contaminants.