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# Silver nanoparticles coated with adenine: preparation, self-assembly and application in surface-enhanced Raman scattering

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**Abstract**
We describe herein the preparation of silver nanoparticles (AgNPs) using nucleobase adenine as protecting agent through the *in situ* chemical reduction of $AgNO_3$ with $NaBH_4$ in an aqueous medium at room temperature. As-prepared AgNPs were characterized by UV–visible spectra, transmission electron microscopy and x-ray photoelectron spectroscopy. All these data confirmed the formation of AgNPs. On the basis of electrostatic interactions between as-prepared AgNPs and anionic polyelectrolyte poly(sodium 4-styrenesulfonate) (PSS), we successfully fabricated (PSS/AgNP)$n$ ($n = 0$–9) multilayers on a 3-mercaptopropyltrimethoxysilane/AgNP functionalized indium tin oxide (ITO) substrate via the layer-by-layer self-assembly technique and characterized as-formed multilayers with UV–visible spectra. Furthermore, these ITO substrates coated with multilayers of different thickness were investigated as surface-enhanced Raman scattering (SERS)-active substrates using $p$-aminothiophenol as a probe molecule, implying that these multilayers substrates may be promising for a new type of SERS-active substrate.

S Supplementary data are available from stacks.iop.org/Nano/18/175610

(Some figures in this article are in colour only in the electronic version)

## 1. Introduction

Owing to both their fundamental and technological interest and importance, noble metal nanoparticles, such as gold, silver, and platinum nanoparticles, have received considerable attention in recent years [1–4]. Among these, a lot of researches have been focused on silver nanoparticles due to their important applications in catalysts [5], anti-microbial agents [6], bioanalysis [7], and surface-enhanced Raman scattering (SERS) substrates [8]. Meanwhile, SERS has been widely studied due to its potential applications in trace analysis [9], nanoscience [10], and biological science [11] since its discovery in the mid-1970s [12]. Thus, much attention has been paid to the metallic colloids, especially silver nanoparticles, as SERS-active substrates because of their strong light scattering and tunable optical properties [13–18].

Quite a number of different stabilizing agents, from the most popular thiol-functionalized organics [19] to polymer [20], polysaccharide [21], and DNA [22], have been used to synthesize silver nanoparticles. Though nucleobase–metal interactions [23] have attracted increasing attention because they hold great promise in antitumour [24] and supra - molecular nano-architecture chemistry [25], there are only a few reports on the preparation of metal nanoparticles using nucleobases and their derivatives as protecting ligands [26]. To the best of our knowledge, no attention has been paid to the preparation of silver nanoparticles coated with adenine nucleobase, although the use of adenine nucleobase as protecting agent for the growth of metal nanostructures is very promising.

In this study, we employed adenine nucleobase as protecting agent to prepare silver nanoparticles (AgNPs) in an aqueous medium by the *in situ* chemical reduction of $AgNO_3$ with $NaBH_4$ in the presence of adenine. The formation of adenine-coated AgNPs was evidenced by UV–visible spectra, transmission electron microscopy (TEM), and x-ray photoelectron spectroscopy (XPS). In addition, the UV–visible studies showed that the as-prepared AgNPs can be fabricated into $(PSS/AgNP)n$ multilayers on a 3-mercaptopropyl-trimethoxysilane/AgNP functionalized indium tin oxide (ITO) substrate via the layer-by-layer (LBL) self-assembly technique [27], and these ITO substrates coated with multilayers of different thickness were investigated as SERS-active substrates using *p*-aminothiophenol (*p*-ATP) as a probe molecule, implying that these multilayers substrates may be promising for a new type of SERS-active substrate.

## 2. Experimental details

### 2.1. Chemicals

Adenine hydrochloride ($C_5H_5N_5{\cdot}HCl$) was obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China). Silver nitrate was purchased from Beijing Chemical Reagent Company (Beijing, China). Sodium borohydride, 3-mercaptopropyltrimethoxysilane (3-MPTMS), poly(sodium 4-styrenesulfonate) (PSS) and *p*-aminothiophenol (*p*-ATP) were purchased from Aldrich (Milwaukee, WI, USA). All reagents were used as received without further purification. The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

### 2.2. Preparation of silver nanoparticles

The adenine-protected silver nanoparticles were prepared according to the following procedure (sample 1): first, 100 $\mu$l silver nitrate aqueous solution (100 mM) and 400 $\mu$l adenine aqueous solution (100 mM) were added together into 10 ml water with initial molar ratio 1:4 of silver nitrate to adenine. 50 $\mu$l sodium borohydride aqueous solution (4.5 mg ml$^{-1}$) was then introduced into the resulting solution under rigorous stirring. The yellowish solution that formed was stored at room temperature for further characterization. Control experiments were carried out as follows: silver nanoparticles was prepared through chemical reduction of silver nitrate aqueous solution with sodium borohydride in the absence of adenine.

To evaluate the influence of the molar ratio of $AgNO_3$ to adenine on the formation of silver nanoparticles, various amounts of adenine were used to prepare the AgNPs, under otherwise identical conditions used for preparing sample 1.

### 2.3. Fabrication of multilayers on ITO substrate via the LBL self-assembly method

For the LBL self-assembly experiment, the transparent ITO glass was pretreated following a reported method [3].

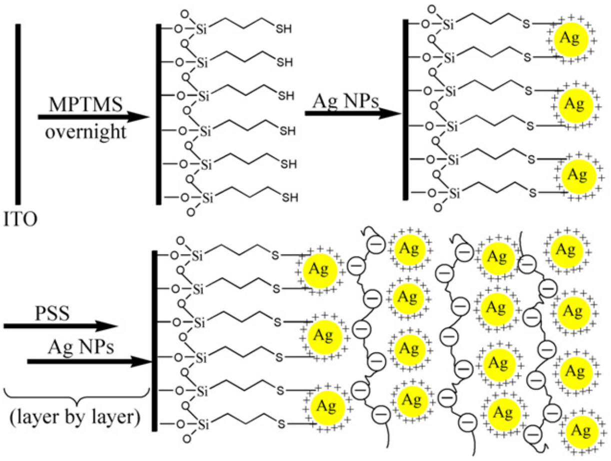

**Figure 1.** Schematic route for the fabrication of $(PSS/AgNP)n$ multilayers on an MPTMS/AgNP functionalized indium tin oxide (ITO) substrate via the layer-by-layer self-assembly technique.

The multilayers of $(PSS/AgNP)n$ on ITO substrate were fabricated as follows (figure 1). Before the assembly of $(PSS/AgNP)n$ multilayers, a precursor film of 3-MPTMS was deposited on a clean ITO surface from its 5% (v/v) methanol solution overnight. After thoroughly rising, the ITO/MPTMS substrate was then immersed in the as-prepared silver colloidal solution for 60 min, giving an ITO/MPTMS/AgNP substrate through metal–thiol interactions. For the construction of $ITO/MPTMS/AgNP/(PSS/AgNP)n$ multilayers ($n = 0$–9) via electrostatic interactions of PSS and AgNPs, the above-mentioned ITO/MPTMS/AgNP substrate was alternately dipped into PSS (1 mg ml$^{-1}$) and as-prepared AgNP aqueous solutions until the desired numbers of layers were obtained (15 min × 2 per cycle). The resulting ITO substrates were used to record UV–visible spectra to follow the self-assembly process.

### 2.4. Preparation of SERS-active substrates

To prepare SERS-active substrates, the ITO substrates coated with $ITO/MPTMS/AgNP/(PSS/AgNP)n$ ($n = 0$–9) multilayers of different thickness were immersed into $1 \times 10^{-4}$ M *p*-ATP ethanol solution for 2 h. Before SERS measurement, the *p*-ATP modified ITO substrates were thoroughly rinsed to get rid of the physically adsorbed probe molecules.

### 2.5. Instrumentation

Absorption spectra were recorded on a Cary 500 Scan UV–visible–NIR spectrophotometer (Varian, Harbor City, CA, USA) at room temperature. Transmission electron microscopy (TEM) measurements were made on a JEOL 2000 transmission electron microscope operated at an accelerating voltage of 200 kV. The sample for TEM characterization was prepared by placing a drop of colloidal solution on a carbon-coated copper grid which was dried at room temperature. Analysis of the x-ray photoelectron spectra (XPS) was performed on an ESCLAB MKII spectrometer (UK) using Mg as the exciting source. The charging calibration was performed by referring

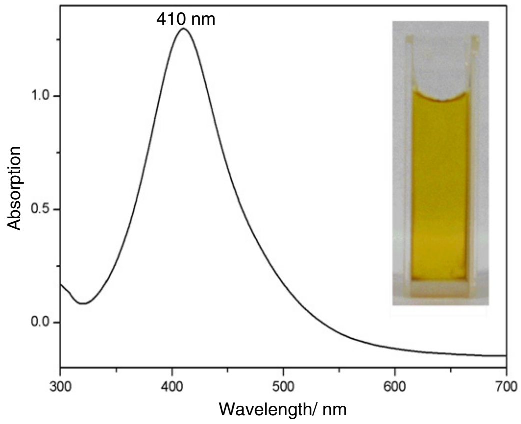

**Figure 2.** UV–visible absorption spectrum and photograph (inset) of silver nanoparticles prepared with initial molar ratio 1:4 of $AgNO_3$ to adenine.

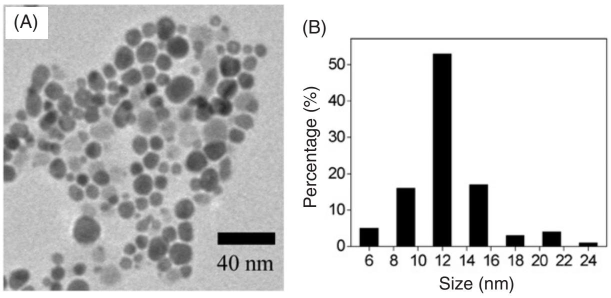

**Figure 3.** Typical TEM image (A) and corresponding particle size distribution histogram (B) of silver nanoparticles obtained with initial molar ratio 1:4 of $AgNO_3$ to adenine.

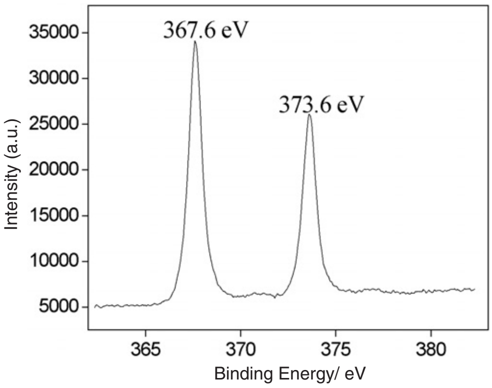

**Figure 4.** XPS spectrum of the Ag 3d peak of the as-prepared silver nanoparticles coated with adenine deposited on the silicon wafer.

the $C_{1s}$ energy level to the binding energy at 284.6 eV. For XPS characterization, the sample was prepared by depositing a drop of as-prepared silver colloidal solution onto a clean silicon wafer and then drying in air.

SERS spectra were recorded with a Renishaw 2000 spectrometer equipped with an Ar+ ion laser giving the excitation line of 514.5 nm and an air-cooled charge coupled device (CCD) as the detector (Renishaw Co., UK). The Raman band of a silicon wafer at 520 $cm^{-1}$ was used to calibrate the spectrometer.

## 3. Results and discussion

### *3.1. Preparation of adenine-coated AgNPs*

Figure 2 shows a typical UV–visible absorption spectrum of the as-prepared silver colloids in water, in which the plasmon absorption band for silver nanoparticles appears at ca 410 nm. This is the characteristic of silver nanoparticles [28]. The yellow tint of the colloidal solution (figure 2, inset) provides further evidence of the formation of the silver particles [29]. This colloidal solution was stable for more than three months without observable aggregation under air conditions, as confirmed by UV–visible analysis. Considering the long-term stability of the silver colloidal solution, we consider that adenine could serve as an effective protective reagent for silver nanoparticles.

The formation of silver nanoparticles was further confirmed by TEM data. Figure 3 depicts a typical TEM image (figure 3(A)) and the corresponding particle size distribution histogram (figure 3(B)) of as-prepared silver nanoparticles, indicating that they were polydisperse, with a diameter of 12 nm. To further confirm the formation of Ag atoms from $AgNO_3$, XPS was used to examine the change in oxidation states for Ag after the reduction reaction had occurred. The XPS spectrum of as-prepared AgNPs shows two peaks centred at binding energy 367.6 and 373.6 eV, which can be assigned to the Ag $3d_{5/2}$ peak and Ag $3d_{3/2}$ peak respectively, confirming the formation of metal Ag (figure 4) [29].

We have also evaluated the influence of the molar ratio of $AgNO_3$ to adenine on the formation of silver nanoparticles by varying the amount of adenine used (figure 5). At 1:1 molar ratio of $AgNO_3$ to adenine, a few nanoparticles with a diameter of 11 nm were formed (some black sediment was observed during the reduction process of $AgNO_3$ due to the relative insufficiency of the protecting agent adenine). At 1:4 molar ratio, we obtained mass products consisting of relative uniform-sized particles with mean diameter 12 nm and a fraction of fused larger particles. However, the 1:10 molar ratio gave products consisting of more fused particles. A further control experiment was carried out in the absence of adenine. As show in figure S1, aggregated silver nanoparticles were obtained in the absence of adenine capping agents (see supplementary information available at stacks.iop.org/Nano/18/175610). The above experimental results clearly suggest that the protecting molecule (i.e. adenine) played a vital role in the stabilization of the AgNPs towards coalescence but did not significantly affect the AgNP size.

### *3.2. Multilayer self-assembly on ITO substrate*

Self-assembly provides an effective and versatile approach to construct ordered and well-defined architectures at the nanoscale [30]. here, we fabricated AgNP-containing multilayers on an ITO glass via the LBL self-assembly technique (figure 1). First, an MPTMS monolayer was fabricated on an ITO substrate via the condensation of

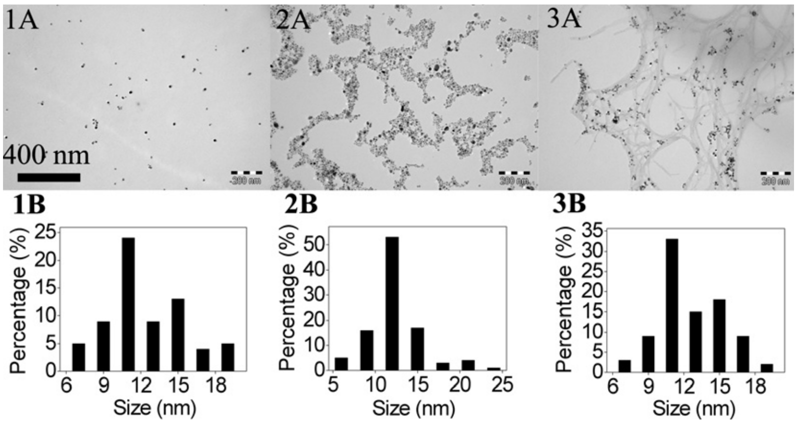

**Figure 5.** TEM images (A) and corresponding particle size distribution histograms (B) of silver nanoparticles obtained with different mole fractions. Initial molar ratio of $AgNO_3$ to adenine = 1:1 (panel 1), 1:4 (panel 2) and 1:10 (panel 3). Scale bar: 400 nm in each case.

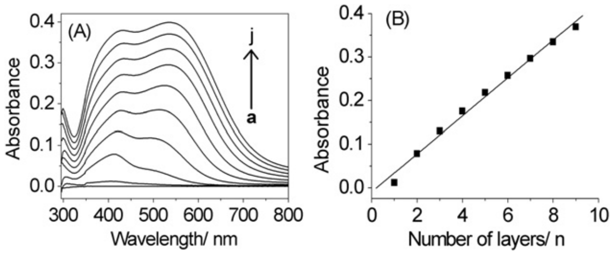

**Figure 6.** (A) UV–visible absorption spectra of ITO/MPTMS/AgNP/(PSS/AgNP)$n$ multilayers with different numbers of PSS/AgNP layers ($n$ = 0–9); (B) the absorbance at 410 nm versus the number of PSS/AgNP layers.

methoxy groups of MPTMS and the silanol groups on the surface of the ITO substrate [31]. The ITO/MPTMS/AgNP was constructed via silver–thiol interactions [32]. Finally, the multilayers of PSS/AgNP were sequentially deposited on the modified ITO/MPTMS/AgNP substrate through the electrostatic interactions of negatively charged PSS and positively charged AgNPs (the AgNPs are positively charged at the surface because the protecting agent adenine is protonated).

Figure 6(A) shows the UV–visible spectra of such a (PSS/AgNP)$n$ multilayer on an ITO/MPTMS/AgNP substrate with increasing $n$. It can be seen that an absorption band of the multilayer-modified ITO substrate occurs at about 410 nm; in addition, a second one begins to appear at about 530 nm and strengthens with sequential assembly. The absorbance at 410 nm corresponding to the surface plasmon resonance band of adenine-coated AgNPs is observed to increase linearly with the number of PSS/AgNP layers (figure 6(B)). This phenomenon indicates that a regular and uniform multilayer film has been structured [3, 33]. The absorbance that begins at around 530 nm should be attributed to the coupled plasmon resonance of silver nanoparticles which are in close contact when the polyelectrolyte PSS and silver nanoparticles are assembled [34]. It is expected that the electrostatic interactions between the negatively charged PSS and positively charged AgNPs played the dominant role in the successful construction of such multilayer structures.

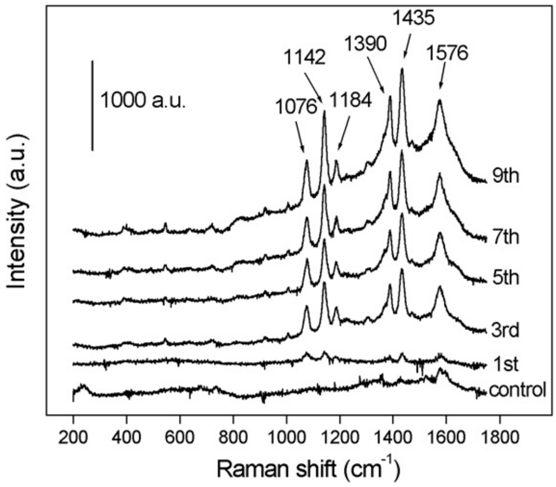

**Figure 7.** SERS spectra of $p$-ATP on modified ITO/MPTMS/AgNP/(PSS/AgNP)$n$ substrate with different number of PSS/AgNP multilayers (control: Raman spectrum of ITO/MPTMS/AgNP without $p$-ATP).

### 3.3. *SERS of p-ATP on the modified ITO substrate*

SERS experiments were conducted to verify that the prepared PSS/AgNP multilayer-modified ITO could be used as a SERS-active substrate. $p$-ATP was chosen as the SERS probing molecule because it is well studied and its thiol group is easily cleaved to form a metal–S bond when adsorbed on a metal surface [8, 35–39]. To investigate the impact of the increasing silver layers on the SERS enhancement, SERS spectra of $p$-ATP were collected at different substrates with various AgNP layers. Figure 7 shows a set of SERS spectra of $p$-ATP on modified ITO/MPTMS/AgNP/(PSS/AgNP)$n$ substrate with various PSS/AgNP multilayers. As indicated in figure 7, the SERS signal intensities increase with the increase of PSS/AgNP multilayers. Based on a rough estimation, the obtained enhancement factor value of the PSS/AgNP multilayers was as large as $6.3 \times 10^4$, which is comparable to the reported enhancement factor value ($1.7 \times 10^5$) [40].

The SERS spectra are dominated by both $a_1$-type vibration modes (in-plane, in-phase modes) at 1076 $cm^{-1}$ and

**Table 1.** Wavenumber positions ($\mathrm{cm}^{-1}$) and assignments for the Raman and SERS spectra of $p$-ATP.

| Solid $p$-ATP[a] ($\mathrm{cm}^{-1}$) | $p$-ATP/the modified ITO substrate[b] ($\mathrm{cm}^{-1}$) | Assignments |
|---|---|---|
| 1591 | | $\nu_{\mathrm{CC}}$, $8a(a_1)$ |
| | 1576 | $\nu_{\mathrm{CC}}$, $8b(b_2)$ |
| 1425 | 1435 | $\delta_{\mathrm{CH}} + \nu_{\mathrm{CC}}$, $19b(b_2)$ |
| 1369 | 1390 | $\delta_{\mathrm{CH}} + \nu_{\mathrm{CC}}$, $3b(b_2)$ |
| 1179 | 1184 | $\delta_{\mathrm{CH}}$, $9a(a_1)$ |
| 1126 | 1142 | $\delta_{\mathrm{CH}}$, $9b(b_2)$ |
| 1084 | 1076 | $\nu_{\mathrm{CS}}$, $7a(a_1)$ |

[a] Assignments for the Raman spectrum of solid $p$-ATP from [8, 37, 38].

[b] Assignments for the SERS spectrum of $p$-ATP on the modified ITO substrate from [8, 36, 37].

1184 $\mathrm{cm}^{-1}$ and $b_2$-type vibration modes (in-plane, out-of-phase modes) at 1576, 1435, 1390, and 1142 $\mathrm{cm}^{-1}$ (also see table 1) [8, 35, 36]. The enhancement of $b_2$-type vibration modes could be attributed to the charge transfer (CT) of the metal to the adsorbed molecules [8, 36, 37]. Meanwhile, the enhancement of $a_1$-type vibration modes should be ascribed to the electromagnetic (EM) field enhancement [8, 39]. The EM field may originate from the strong inter-nanoparticle coupling among close-contacted AgNPs assembled on the ITO substrate [35]. This is consistent with the result obtained from the UV spectra evolution of the assembled multilayers on the ITO substrate, where a 530 nm absorption band appeared as a consequence of the AgNPs fabricating into the multilayers (figure 6(A)) [34].

## 4. Conclusions

In summary, stable, nucleobase-protected silver nanoparticles dispersed in water medium can be easily prepared by the *in situ* chemical reduction of $AgNO_3$ in adenine aqueous solution. The formation of silver nanoparticles coated with adenine was well characterized, and it has been proven that adenine could serve as an effective protective reagent for silver nanoparticles. The as-prepared silver nanoparticles have also been successfully fabricated into multilayers on ITO substrate via an alternative layer-by-layer assembly technique with negatively charged polyelectrolyte PSS. The PSS/AgNP multilayer-modified ITO substrate has been demonstrated to be a SERS-active substrate for probing $p$-ATP.

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