

Ascorbic acid-functionalized Ag NPs as a probe for colorimetric sensing of glutathione

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Abstract In this work, we report the use of ascorbic acid-capped silver nanoparticles (AA–Ag NPs) as a probe for selective colorimetric detection of glutathione (GSH) in aqueous solution. This detection system was based on the GSH-induced aggregation of AA–Ag NPs, resulting in drastic changes in the absorption spectra and color of the AA–Ag NPs system. The GSH-induced AA–Ag NPs aggregation was confirmed by UV–visible spectrometry, dynamic light scattering (DLS) and transmission electron microscopic (TEM) techniques. Under optimal conditions, this method exhibited good linearity over the concentration ranges from 5.0 to 50 μM , with the limit of detection 2.4×10^{-7} M. This method was successfully applied to detect GSH in the presence of other biomolecules, which confirms that this probe can be used for the detection of GSH in real samples.

Keywords AA–Ag NPs · GSH · UV–visible spectrometry · DLS and TEM

Introduction

Glutathione (GSH, γ -glutamylcysteinylglycine) is a primary non-protein sulfhydryl in most of the cells. It contains an unusual peptide linkage between γ -carboxyl group of Glu side chain and amino group of cysteine (Valko et al.

2007). It plays a key role in maintaining redox homeostasis in cells and acts as an antioxidant for keeping the thiol group of cysteine in proteins (Dröge 2002). The reduced glutathione/oxidized glutathione ratio is used to evaluate oxidative stress in cells. It serves as a cofactor for glutathione peroxidase in the decomposition of hydrogen peroxide or organic peroxides and for glyoxalase 1 in the detoxification of methylglyoxal and other α -oxo-aldehydes (Metzler 2004). Furthermore, it plays a significant role in studying various diseases such as HIV, diabetes, liver damage, heart diseases, mild cognitive impairment, edema, pneumonia and skin lesions (Droge and Holm 1997; Staal 1998; Micke et al. 2001; Julius et al. 1994; Bray and Taylor 1994). Therefore, it is highly desirable to develop a new sensitive, fast and practical method for the detection of GSH in biological samples. Although conventional analytical tools including electrospray ionization mass spectrometry (ESI–MS) (Kailasa et al. 2012), matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) (Wan et al. 2013), high-performance liquid chromatography (HPLC) (Casey et al. 2002), fluorescence spectrometry (Shao et al. 2010) and electrochemistry (Safavi et al. 2009) could meet the demand for the sensitive and selective detection of GSH in biological samples, all these methods are expensive, time-consuming and require tedious sample preparations. In this frame, it is necessary to develop a facile and sensitive method for the analysis of GSH in various samples.

The integration of nanotechnology with analytical chemistry is becoming increasingly important for the development of miniaturized analytical tools for ultrasensitive detection of various target analytes including biomolecules and inorganic and organic molecules (Kailasa and Wu 2013, Kailasa et al. 2013; Vilela et al. 2012). Metallic nanoparticles (Au and Ag NPs) have attracted

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extensive research interest as a promising colorimetric probe for sensing different target analytes in the past two decades, due to their easy preparation and unique optical properties [surface plasmon resonance absorption (SPR) and resonance light scattering] (Jiang et al. 2011). As a result, many functionalized Ag NPs have been used as colorimetric sensors for the detection of a wide variety of molecules including organic (Tashkhourian et al. 2011; Zhang et al. 2010), inorganic (Mehta et al. 2013; Modi et al. 2014; Yao et al. 2010) and biomolecules (Ravindran et al. 2011; Yuan et al. 2013). These methods are based on the analytes-induced aggregation of Ag NPs, resulting in changes in both color and absorption spectrum. These analytes-induced aggregations are due to the interactions between surfaces of Ag NPs and analytes, confirming that the surface modification of NPs is very important to

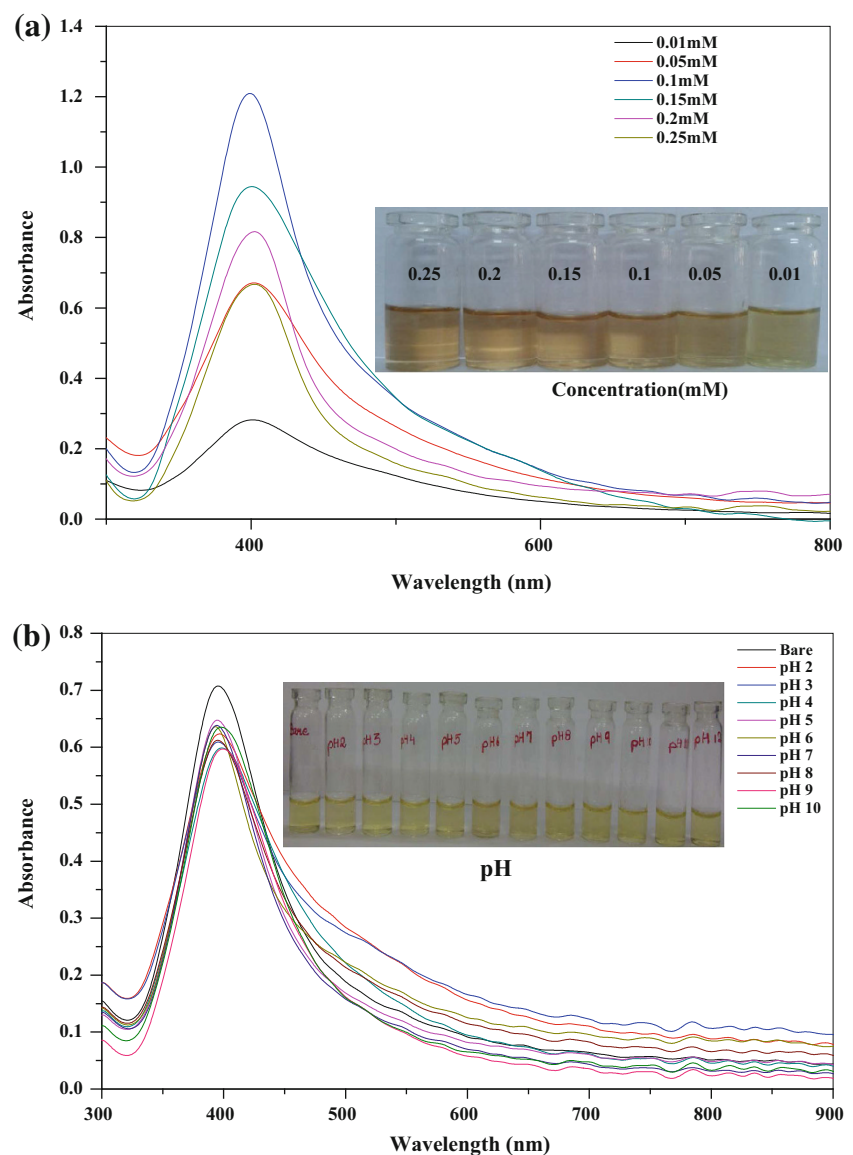
improve the stability and sensing ability of Ag NPs. So far, the use of ascorbic acid-functionalized Ag NPs as a colorimetric sensor for the detection of GSH has been unexplored. Herein, we report a novel colorimetric method for the detection of GSH using ascorbic acid-functionalized Ag NPs as a colorimetric probe.

Materials and methods

Chemicals and materials

Silver nitrate, sodium borohydride, ascorbic acid, sodium acetate and glutathione were purchased from Sigma-Aldrich, USA. Threonine, histidine, glutamic acid, serine, lysine HCl, cysteine, tyrosine and tryptophan were

Fig. 1 **a** UV–visible spectra of Ag NPs using ascorbic acid (0.01–0.25 mM) as a capping agent. **b** UV–visible spectra of AA–Ag NPs at different sodium acetate buffer pH values from 2 to 10



purchased from SRL Chemicals. Milli-Q-purified water was used for the sample preparations.

Synthesis of AA–Ag NPs

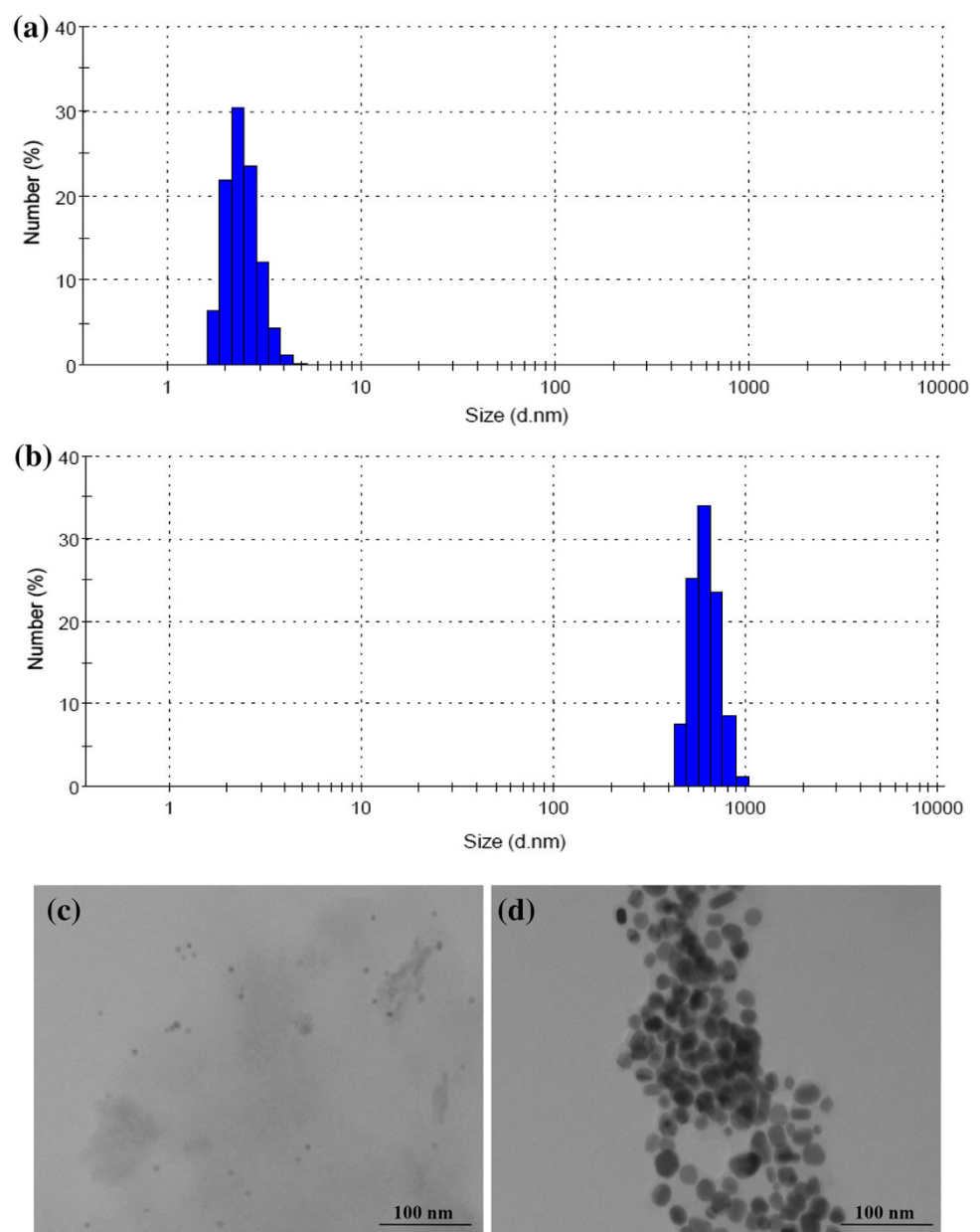
Ag NPs were prepared according to the method in the literature (Pinto et al. 2010) with minor modifications. Briefly, 5 mL of 0.25 mM silver nitrate was taken into a 50-mL reaction flask that contained 5 mL of 0.1 mM ascorbic acid and then stirred for 30 s. To this, 0.5 mL of 1.5 mM NaBH₄ was added dropwise and stirred for 10 min, resulting in a color change from colorless to light yellow, which confirms the formation of AA–Ag NPs. Supporting information of Scheme 1 shows the

schematic representation for the preparation of AA–Ag NPs.

Detection of GSH

For detection of glutathione using AA–Ag NPs, different concentrations of GSH (5.0–50 μ M, 0.1 mL) were added separately into sample vials that contained 1 mL of AA–Ag NPs at sodium acetate buffer pH 7.0. As a result, the color of AA–Ag NPs was changed from bright yellow to orange-brown color, indicating that the aggregation of AA–Ag NPs was induced by GSH. Subsequently, their absorption spectra were measured by UV–visible spectrometer.

Fig. 2 DLS of **a** AA–Ag NPs and **b** GSH-induced aggregation of AA–Ag NPs. TEM images of **c** AA–Ag NPs and **d** GSH-induced aggregation of AA–Ag NPs



Instrumentation

Absorption spectra were recorded on a Maya Pro 2000 spectrophotometer, Ocean Optics, USA. Dynamic light scattering (DLS) data were measured using Zetasizer Nano ZS90 (Malvern, UK). Fourier transform-infrared (FT-IR) spectra were recorded using FT-IR 8400S (Shimadzu, Japan) with KBr powder. Transmission electron microscopy (TEM) images were taken using CM200 (Philips, Netherlands).

Results and discussion

Characterization of AA–Ag NPs

The synthesized AA–Ag NPs were characterized by FT-IR, DLS, TEM and UV–visible spectroscopy. To improve the sensing ability of Ag NPs, we optimized the concentrations of NaBH_4 as a reducing agent and AA as a capping agent. As shown in Fig. 1a and supporting information of Fig.

S1a, the best absorption spectrum of AA–Ag NPs was obtained using 1.5 mM of NaBH_4 as a reducing agent and 0.1 mM of AA as a capping agent. It can be observed that there is no obvious change in the SPR band and a characteristic SPR band at 397 nm exists, which suggest that the AA–Ag NPs have the optical property of the original Ag NPs. We also studied the effect of reaction temperature on the formation of Ag NPs. It was noticed that the maximum absorbance (at 397 nm) and intense yellow color were observed by performing the reaction at room temperature (supporting information of Fig. S1b). Supporting information of Fig. S2 shows the FT-IR spectra of pure AA and AA–Ag NPs. The peaks at 3,526 and 3,412–3,216 cm^{-1} were not observed in the FT-IR spectrum of AA–Ag NPs, which confirms the oxidation of hydroxy groups in AA. The pure AA spectrum showed peaks at 3,212–3,626 cm^{-1} , which corresponded to stretching of the –OH groups. The peaks at 3,030 and 2,879 cm^{-1} corresponded to the asymmetric and symmetric vibrations of – CH_2 group. The carbonyl group (C=O) stretching was observed at $\sim 1,760 \text{ cm}^{-1}$ and the vibration

Fig. 3 UV–visible spectra of **a** AA–Ag NPs in the presence of GSH over different amino acids (threonine, lysine, serine, cysteine, histidine, glutamic acid, tyrosine, tryptophan) and **b** photographic image of AA–Ag NPs in the presence of various amino acids

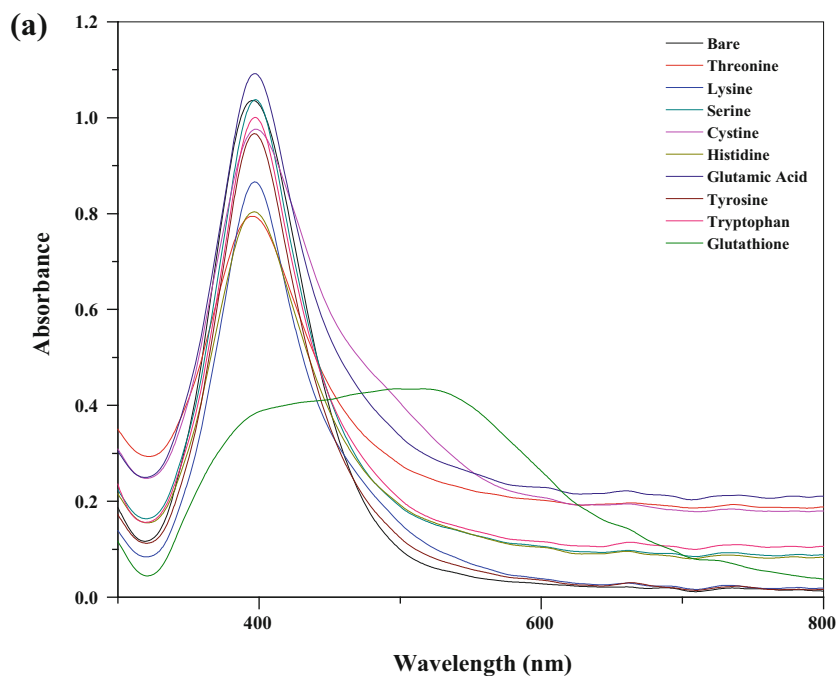
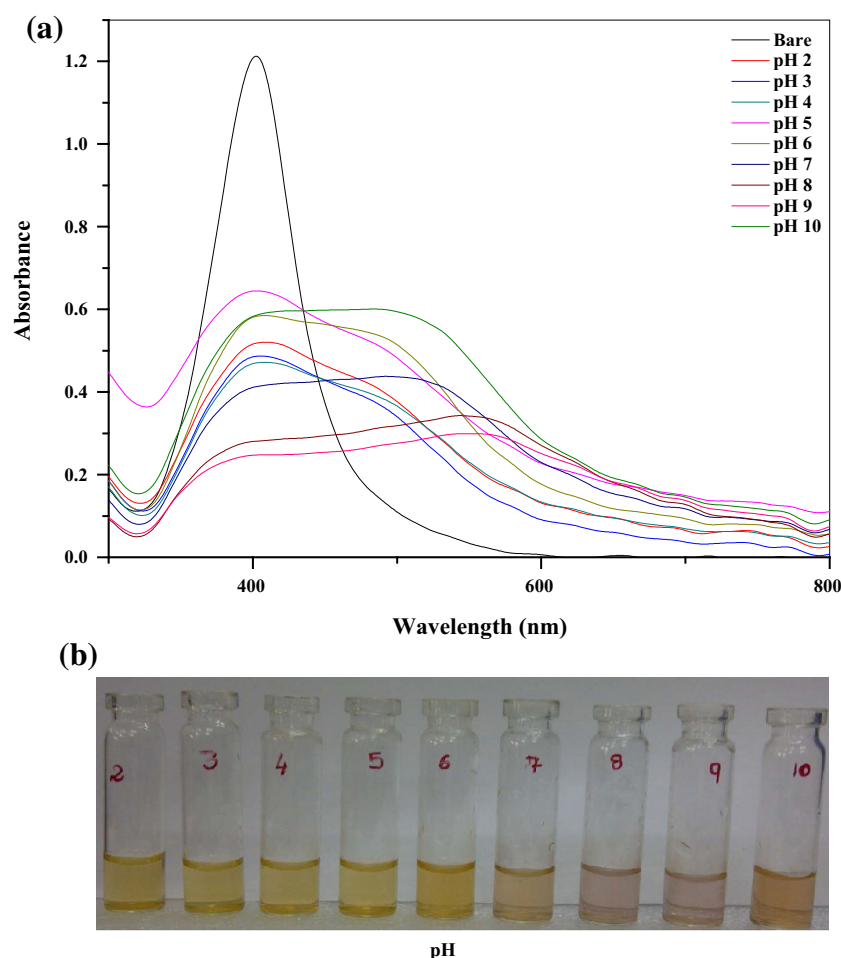


Fig. 4 a UV–visible spectra of AA–Ag NPs by the addition of GSH at sodium acetate buffer pH values from 2.0 to 10.0. **b** Photographic image of the corresponding solutions



frequency of C=C was observed at $\sim 1,670\text{ cm}^{-1}$. It can be observed that the –OH group stretching disappeared at $3,212\text{--}3,626\text{ cm}^{-1}$, which indicates that AA molecules successfully interacted with Ag NPs. Figure 2a and c shows the DLS and TEM image of AA–Ag NPs, which indicate that AA–Ag NPs are well dispersed in water with uniform size. The average diameter of AA–Ag NPs is 2.5 nm.

AA–Ag NPs as a probe for colorimetric sensing of GSH

To investigate AA–Ag NPs as a colorimetric sensor, various biomolecules (threonine, histidine, glutamic acid, serine, lysine HCl, cysteine, tyrosine, tryptophan and GSH, 0.1 mL, 1 mM) solutions were added into 1 mL of AA–Ag NPs solutions with sodium acetate buffer pH 7.0. As shown in Fig. 3, the characteristic SPR peak of AA–Ag NPs at 397 nm is redshifted to 468 nm only by the addition of GSH, resulting in a color change from yellow to orange-brown, which confirms that the aggregation of AA–Ag NPs was induced by GSH. To investigate the effect of pH condition on the absorption spectra of AA–Ag NPs, we

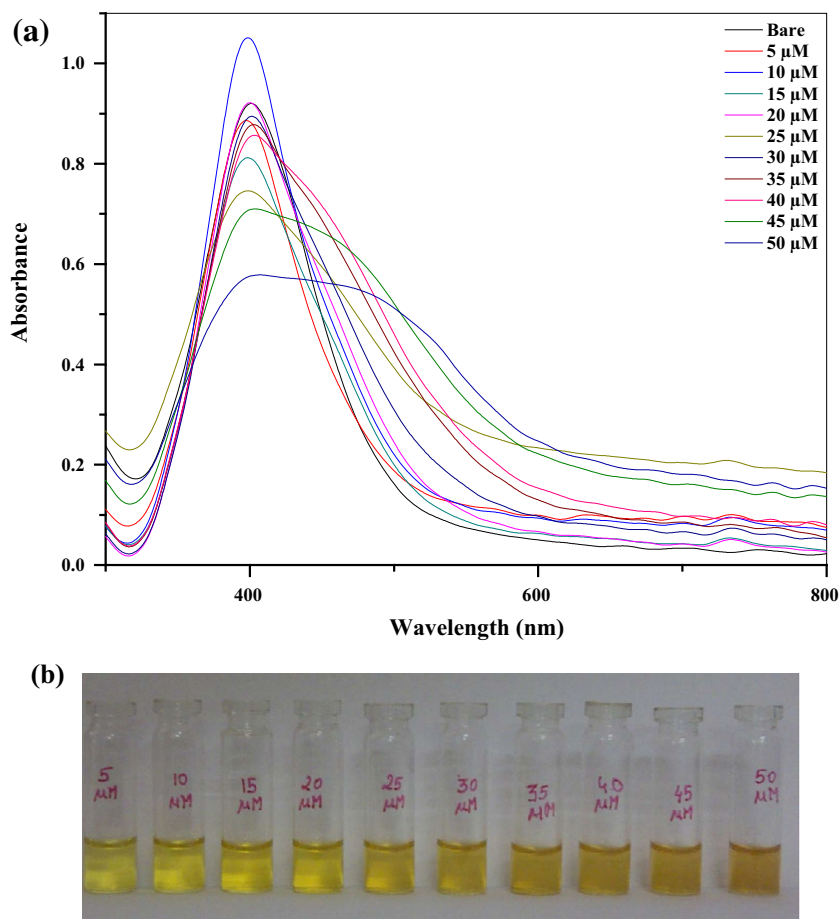
measured UV–visible spectra of AA–Ag NPs with sodium acetate buffer (pH 2.0 to 10.0) without GSH. As shown in Fig. 1b, the UV–visible spectra of AA–Ag NPs are drastically changed at lower pH of 2.0–4.0, which is due to the surface neutralization of AA–Ag NPs. At the same time, UV–visible absorption spectrum of AA–Ag NPs remained the same at pH 7.0, which suggests that the color and absorption spectrum of AA–Ag NPs did not influence sodium acetate buffer pH at pH 7.0. To investigate the effect of pH on GSH-induced aggregation of AA–Ag NPs, we studied the UV–visible spectra of AA–Ag NPs upon the addition of GSH with sodium acetate buffer pH in the range of 2.0–10.0 (Fig. 4). With sodium acetate buffer pH 7.0, the characteristic SPR band of AA–Ag NPs at 397 nm is redshifted toward longer wavelength (468 nm), resulting in a color change from yellow to orange-brown, which confirms the aggregation of AA–Ag NPs induced by GSH. AA–Ag NPs (AA–pKa 4.10) surfaces and GSH (pI = 5.90) have exhibited negative and positive charges, which is due to their pI values. As a result, there is strong electrostatic interaction between AA–Ag NPs and GSH. Furthermore, GSH strongly induced the aggregation of

AA–Ag NPs because of the stronger affinity of GSH toward AA–Ag NPs, due to the multidentate anchoring groups (e.g., –SH, –NH₂ and –COO[−]) of GSH. Therefore, GSH effectively induced the aggregation of AA–Ag NPs via multiple interactions (electrostatic and covalent), resulting in changes in both UV–visible spectra and color of the AA–Ag NPs. To confirm the GSH-induced AA–Ag NPs aggregation, we measured the DLS and TEM of AA–Ag NPs in the presence of GSH (Fig. 2b, d). It can be observed that AA–Ag NPs sizes increased to 619.5 nm, which confirms that the aggregation of AA–Ag NPs was induced by GSH. Supporting information of Fig. S3 shows the absorbance ratio at $A_{468\text{nm}}/A_{397\text{nm}}$ that can describe the degree of aggregation ability of GSH with AA–Ag NPs. Based on the above results, AA–Ag NPs acted as a selective colorimetric probe for sensing of GSH in aqueous solution.

To construct the calibration graph for GSH analysis, we measured the UV–visible spectral changes of AA–Ag NPs by the addition of different concentrations of GSH from 5.0 to 50 μM (Fig. 5a). It can be observed that the new SPR peak intensity was gradually increased with increase in the concentration of GSH, which confirms that the degree of GSH-induced AA–Ag NPs aggregation was

increased with increase in the concentration of GSH up to 50 μM , and resulted in a redshift in wavelength (from 397 to 468 nm) and a color change from yellow to orange-brown (Fig. 5b). Under optimal conditions, a linear relationship ($R^2 = 0.997$) exists between the absorption ratio ($A_{468\text{nm}}/A_{397\text{nm}}$) and the concentration of GSH over the range of 5.0 and 50 μM with the limit of detection 2.4×10^{-7} M (supporting information of Fig. S4). To evaluate the sensing capability of AA–Ag NPs in the presence of other biomolecules, we studied the absorption spectra of AA–Ag NPs upon the addition of GSH in the presence of difference molecules (threonine, histidine, glutamic acid, serine, lysine HCl, cysteine, tyrosine, and tryptophan, 0.1 mL, 1 mM) and the obtained spectra are shown in supporting information of Fig. S5. It was noticed that the AA–Ag NPs system successfully detected the GSH molecules in the presence of other similar molecules, which represents that the developed system could not produce any wrong signal in the presence of other similar analytes. These results suggest that AA–Ag NPs-based sensor exhibited high selectivity to detect GSH in the presence of other threonine, histidine, glutamic acid, serine, lysine HCl, cysteine, tyrosine and tryptophan molecules.

Fig. 5 **a** UV–visible spectra of AA–Ag NPs by the addition of GSH in the range of 5.0–50 μM . **b** Photographic image of the corresponding solutions



Conclusions

In summary, we have developed a facile and selective colorimetric method for detection of GSH using AA–Ag NPs. The GSH-induced AA–Ag NPs aggregation results in a noticeable redshift (from 397 to 468 nm) in the UV–visible absorption spectrum and a visible color change from yellow to orange-brown, which allow the detection of GSH with a detection limit of 2.4×10^{-7} M. The AA–Ag NPs acted as a colorimetric sensor for the detection of GSH in the presence of other similar molecules. This method provides a simple platform for the detection of GSH in biological samples with high selectivity and sensitivity.

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