Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Au@Pt bimetallic nanoparticles and blue silica nanoparticles nanocomposites as probes of immunochromatographic assay for HBsAg detection

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Chemicals and materials

Tetraethyl orthosilicate (TEOS, 98%), Ethanol, ammonia ($NH_3 \cdot H_2O$, 25-28 wt%) and ascorbic acid were received from Aladdin Industrial Inc. (Shanghai, China). C.I. Reactive Blue 194 was purchased from Zhejiang Shunlong Chemical Co., Ltd. (Zhejiang, China). Carboxyethylsilanetriol sodium salt 25 wt% in water (CES) was obtained from J&K Scientific Co., Ltd (Shanghai, China). Trisodium citrate was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Chloroplatinic acid hexahydrate (H_2PtCl_6) was received from Aladdin Industrial Inc. (Shanghai, China). Chloroauric acid hydrate ($HAuCl_4$) was purchased from Hangzhou Chemical Reagent Co.Ltd.(Hangzhou, China). The diagnostic kits for detection of HBsAg (based on AuNPs) was received from Artron Laboratories Inc. (Burnaby, Canada). All others chemical reagents were of analytical grade. All the solutions were prepared with ultra-pure water, which is produced by Elix-3 and Milli-QA (Molsheim, France).

Instruments

The Thermo Nicolet 380 Fourier transform infrared spectrometer (FTIR) was provided by Thermo Fisher Scientific Co. (Shanghai, China).

Synthesis of blue SiNPs

Blue SiNPs were prepared according to our previously reported method. Briefly, 20 μL of 3APTMS and 80 μL of C.I. Reactive Blue 194 aqueous solution (0.1 g/mL) were added into 15 mL of water containing 0.1g silica nanoparticles to react 2.5 h under magnetic stirring. The blue SiNPs were isolated by centrifugation (8,000 rpm, 3 min), and washed with ethanol and water respectively. A Stöber system containing 10 mL ethanol, 815μL H_2O, 200μL TEOS, and 100μL $NH_3 \cdot H_2O$ (25-28%) was hydrolyzed. After stirring within 30 min, 2.5 mL of above prepared blue

SiNPs ethanol dispersion solution was added into the pre-hydrolyzed Stöber system and react for 12 h at room temperature under magnetic stirring. The blue SiNPs were purified by centrifugation (10,000 rpm, 5 min) and washed with ethanol and water respectively.

Synthesis of blue SiNPs-COOH

After that, 25 μL of CES was added into the Stöber system to stir another 24 h. The final core-shell purple SiNPs were isolated by centrifugation and washed three times with ethanol and water, finally dispersed in pure water for further usage.

Synthesis of Au@Pt nanospheres

Au@Pt nanospheres were prepared according to our previously reported method. First, 0.5 mL of 1 wt % $HAuCl_4$ solution was added to 50 mL of aqueous solution and the solution was heated to boiling with stirring. Then 0.75 mL of 1 wt % sodium citrate was quickly introduced to the above solution. After heating for several minutes, the golden-red solution appeared, indicating the formation of gold nanospheres. Then 1 mL of 0.1 M ascorbic acid (excess) was subsequently added to the gold NP boiling solution, followed by adding 0.625 mL of 1 wt % H_2PtCl_6. In this process, the heat-treatment could obviously accelerate the kinetics of reduction of Pt salt by ascorbic acid on the colloidal gold surface. After 20 min of heating, the urchinlike Au-Pt NPs were obtained (30 mL).

Preparation of antibody-modified blue SiNPs conjugate

The mouse anti-HBsAg monoclonal antibody (1.7 mg/mL) was immobilized on the surface of blue SiNPs through carbodiimide chemistry with slight modification. Briefly, 1 mg of the blue SiNPs were diluted in MES (0.05 M, PH 6.0) with a total volume of 1 mL. 2 mg EDC and 3 mg NHS were added to the solution and allowed to react for 15 min with gentle mixing to activate the

carboxyl group on the blue SiNPs. The activated nanoparticles were washed twice to remove unreacted chemicals and reconstituted in 1 mL of phosphate buffer (PB, 0.02 M, pH 7.4). 10 μg mAb1 was added into the above solution and mixed gently for 2.5 h at room temperature. The conjugates were centrifuged and suspended in 1 mL of 2% BSA (w/v) in PB to block unreacted sites for 1 h at room temperature. The blocked conjugates were centrifuged and suspended in phosphate buffered saline (PBS) containing 1% BSA (w/v), 5% sucrose (w/v) and 3% trehalose (w/v) at a final concentration of 10 mg/mL and stored at 4℃ before use.

Characterization of blue SiNPs-NH_2

The presence of amino group on the outermost layer of blue SiNPs was confirmed by the FT-IR spectrograms. Dried samples were measured using KBr pellet method in the range of 500-4000 cm^{-1}. As depicted in Fig., As shown in FTIR spectra of blue SiNPs-NH_2, new bands at 1407 cm^{-1}, 1434 cm^{-1} and 1526 cm^{-1} are attributed to the N-H bonds of amino groups on the silica surface,indicating the success of amino-functionalization.

The optical property of blue SiNPs-NH_2, Au@Pt and Au@Pt/blue SiNPs

The optical property of nanoparticle is strongly dependent on the composition of the nanoparticle. The nanocomposite offers a new routine to merge the merit of each independent composite metal material in regard to optical and chemical properties. The UV-vis absorption spectra of the blue SiNPs suspension, Au@PtNPs suspension, and Au@Pt/blue SiNPs suspension are presented in Fig. 2s. There is no absorption peak for Au@Pt NPs in the full-wave band (Fig.2s, a), while there is peak at 620 nm for blue SiNPs (Fig.2s, c). Au@Pt/blue SiNPs suspension shows no obvious absorption peak (Fig.2s, b), probably because its surface is covered with a large amount of Au@Pt NPs. The change of the absorption peak of the nanocomposite may be due to

both the size difference and the interaction between Au@Pt NPs and blue SiNPs.

Comparison with traditional colloidal gold-based ICA

According to the previous definition, it can be seen that the traditional colloidal gold-based ICA has a visual detection limit (VDL) of 5 ng/mL. Therefore, the sensitivity of the new immunochromatographic test established in this experiment was improved at least 10-fold compared to traditional colloidal gold-based ICA .

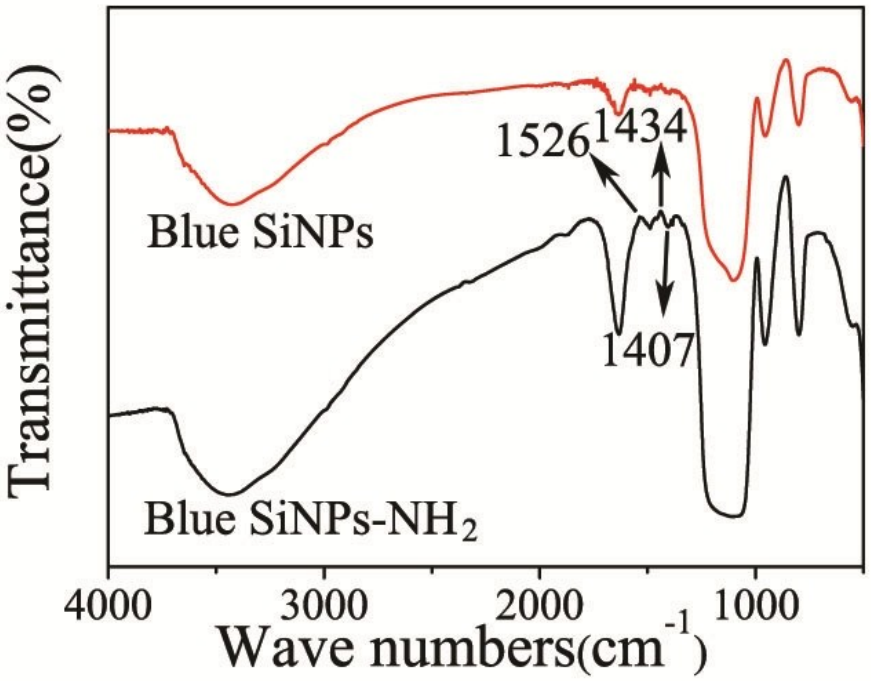

Fig. 1S. The FTIR spectra of blue-SiNPs and blue-SiNPs-NH_2

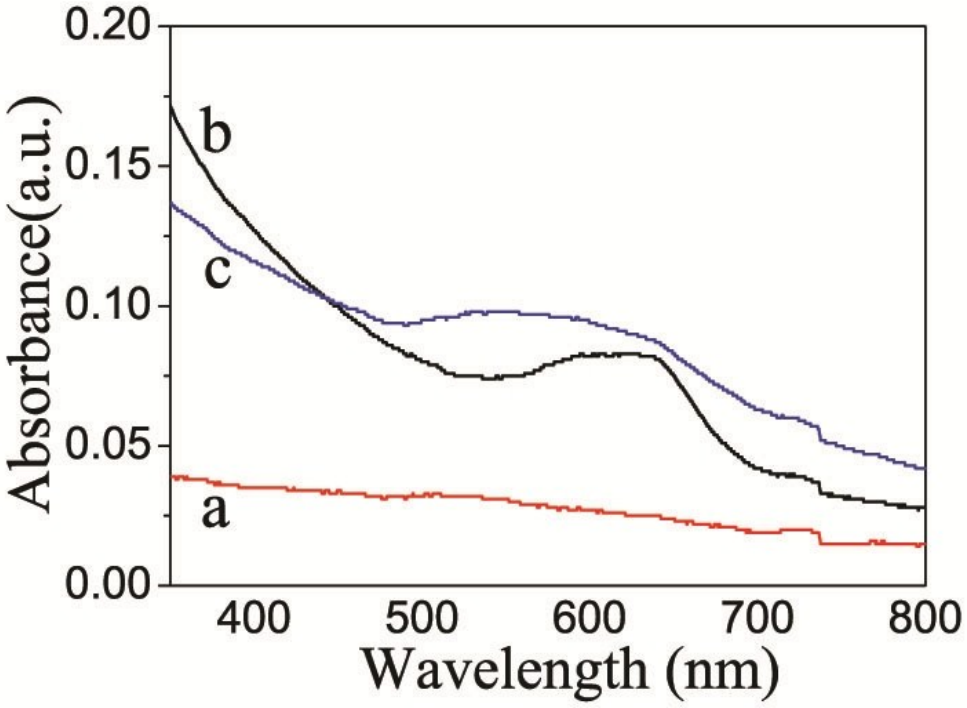

Fig. 2S. UV-vis spectra of the three kinds of colloidal solutions: Au@AgNPs(a), Au@Pt/blue SiNPs composites(b), blue-SiNPs(c).

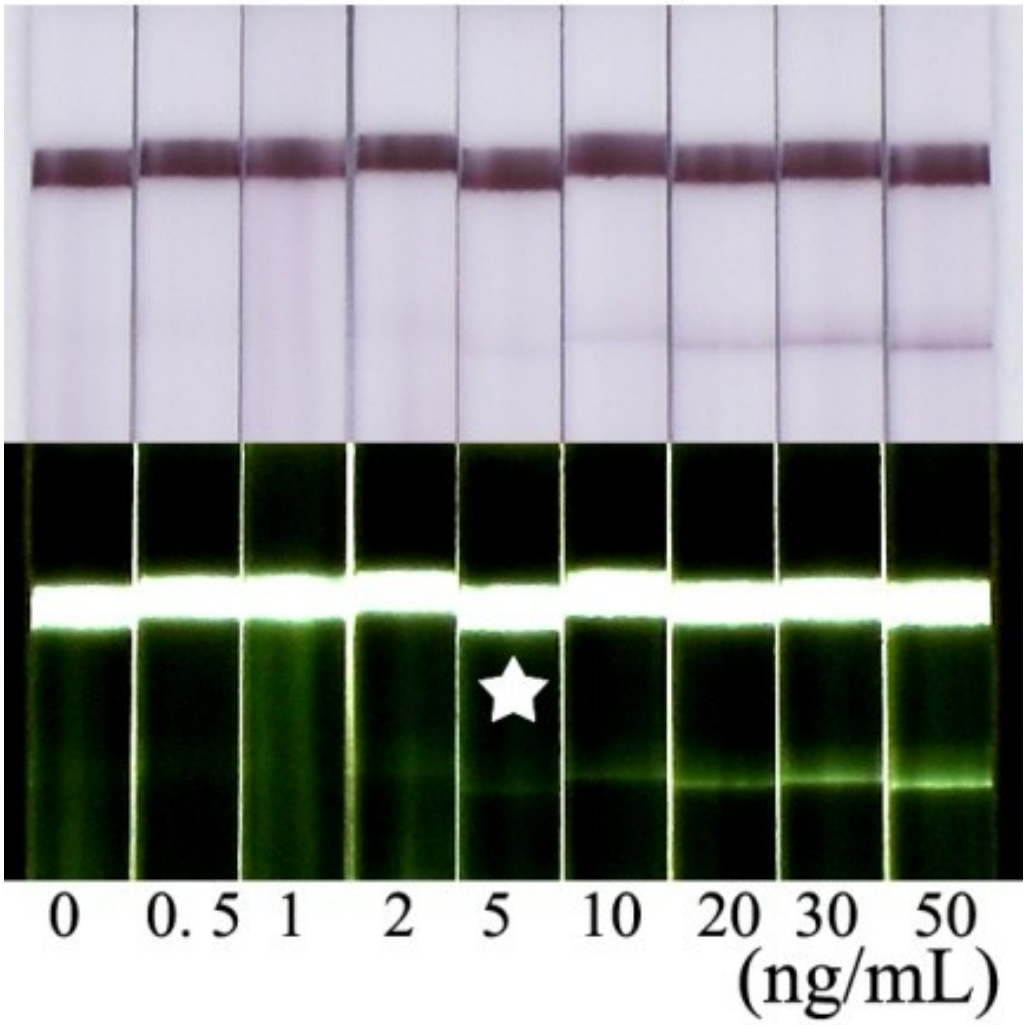

Fig. 3S The color photographs (up) and contrast enhanced black and white negative images (down) of the AuNPs-based ICA strips after detection of HBsAg in serum samples.