Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Highly precise plasmonic and colorimetric sensor based on enzymatic etching of nanospheres for the detection of blood and urinary glucose

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Experimental details

Materials. Hydrogen tetrachloroaurate (III) hydrate was purchased from Kojima Chemicals Co. (Sayama, Saitama, Japan). Silver nitrate, ethylene glycol, polyvinylpyrrolidone (MW 40 kD), glucose, glucose oxidase, citric acid were purchased from Sigma (St.Louis, MO, USA). Trisodium citrate and hydrogen peroxide (30%) were purchased from Junsei (Tokyo, Japan). All chemicals were used as received.
Healthy human blood and urine were gathered in Seoul National University Medical Health Center under the permission of Institutional Review Board (IRB No. 1408/001-014)

Synthesis of PVP-AgNPs and PVP-AuAg

Synthesis of 100 nm PVP-AgNPs

First, PVP (500 mg) was dissolved in ethylene glycol (30 mL) by sonication and vortexing. The prepared PVP solution was heated to 170 ℃ followed by addition of 480 mg of silver nitrate dissolved 30 mL of ethylene glycol solution. The reaction mixture was boiled for 2 hrs with vigorous stirring and then cooled to room temperature. The product was rinsed with distilled water 4 times using centrifugation at 8000 rpm for 20 min each by Centrifuge 5810R (Eppendorf, Germany). Finally, PVP-AgNPs were redispersed in 200 mL of distilled water, and stored in dark.

Preparation of PVP-AuAg by galvanic replacement reaction

To 1 mL of the prepared 250 pM PVP-AgNPs, 20 μL of 1 mM hydrogen tetrachloroaurate (III) hydrate stock was added. After addition of Au (III) solution, the mixed solution was simply inverted and incubated for 10 min with shaking. When the color change finished, the mixture was rinsed with distilled water 4 times using centrifugation at 8000 rpm for 20 min each. The final product was characterized by UV-Vis spectrophotometry and stored at room temperature.

Characterization of PVP-AgNP and PVP-AuAg before and after etching

Energy-filtering transmission electron microscope LIBRA 120 (Carl Zeiss, Germany) was used to obtain images of PVP-AuAg before and after the etching reaction. UV-Vis spectrophotometer S-3100 (Scinco, Korea) was used to obtain UV-Vis absorption spectra during the enzymatic reaction.

Measurement of plasmonic peak shift by using a UV-Vis spectrophotometer

Plasmonic peak shift according to the amount of added hydrogen peroxide in distilled water

To 150 pM of 2 mL PVP-AuAg in distilled water, designated amount of hydrogen peroxide was added to make final concentrations between 0 to 100 μM. The mixture was incubated in quartz cuvette for 10 min at ambient condition and then, absorption spectra were measured by using a UV-Vis spectrophotometer.

Plasmonic peak shift according to the GOx reaction in buffer solution

To 150 pM of 2 mL PVP-AuAg in pH 5.1 citrate buffer solution (20 mM) containing designated amount of glucose between 0 to 250 μM, GOx was added with final concentration of 100 nM. The absorption spectra of the resulting mixture were recorded by using a UV-Vis spectrophotometer after 15 min of incubation at ambient condition.

Plasmonic peak shift according to the GOx reaction in 1:50 diluted human serum

Human serum was centrifuged using an Amicon Ultra filter with a 3,000 Da cutoff to remove glucose. After filtering, the serum was spiked with different concentrations of glucose between 1 to 10 mM. The dilution factor of the serum in the assay was 1:50 in pH 5.1 citrate buffer solution (20 mM) containing 150 pM of PVP-AuAg, to make final concentration after dilution become 1 to 200 μM. Finally, 100 nM of GOx was added to the prepared mixture and the absorption spectra of the resulting mixture were recorded after 15 min of incubation at ambient condition.

Plasmonic peak shift according to the GOx reaction in 1:50 diluted human urine

Human urine was centrifuged using an Amicon Ultra filter with a 3,000 Da cutoff to remove glucose. After filtering, the urine was spiked with 5 and 10 mM glucose. The dilution factor of the serum in the assay was 1:50 in pH 5.1 citrate buffer solution (20 mM) containing 150 pM of PVP-AuAg, to make final concentrations after dilution become 100 and 200 μM, respectively. Finally, 100 nM of GOx was added to the mixture and the absorption spectra of the resulting mixture were recorded after 15 min of incubation under ambient condition.

Plasmonic peak shift according to the GOx reaction of human blood samples obtained before and 2 hr after a meal

Human blood was drawn before and 2 hr after a meal into vacutainer (Becton Dickinson). After collection of the whole blood, the blood was allowed to clot by leaving it undisturbed at room temperature for 30 min. Then, we removed the clot by centrifuging at 1000g for 10 min in a refrigerated centrifuge. The dilution

factor of the serum in the assay was 1:50 in pH 5.1 citrate buffer solution (20 mM) containing 150 pM of PVP-AuAg, to make final concentration after dilution become 100 and 200 μM, respectively. Finally, 100 nM of GOx was added to mixture and the absorption spectra of the resulting mixture were recorded after 15 min of incubation under ambient condition.

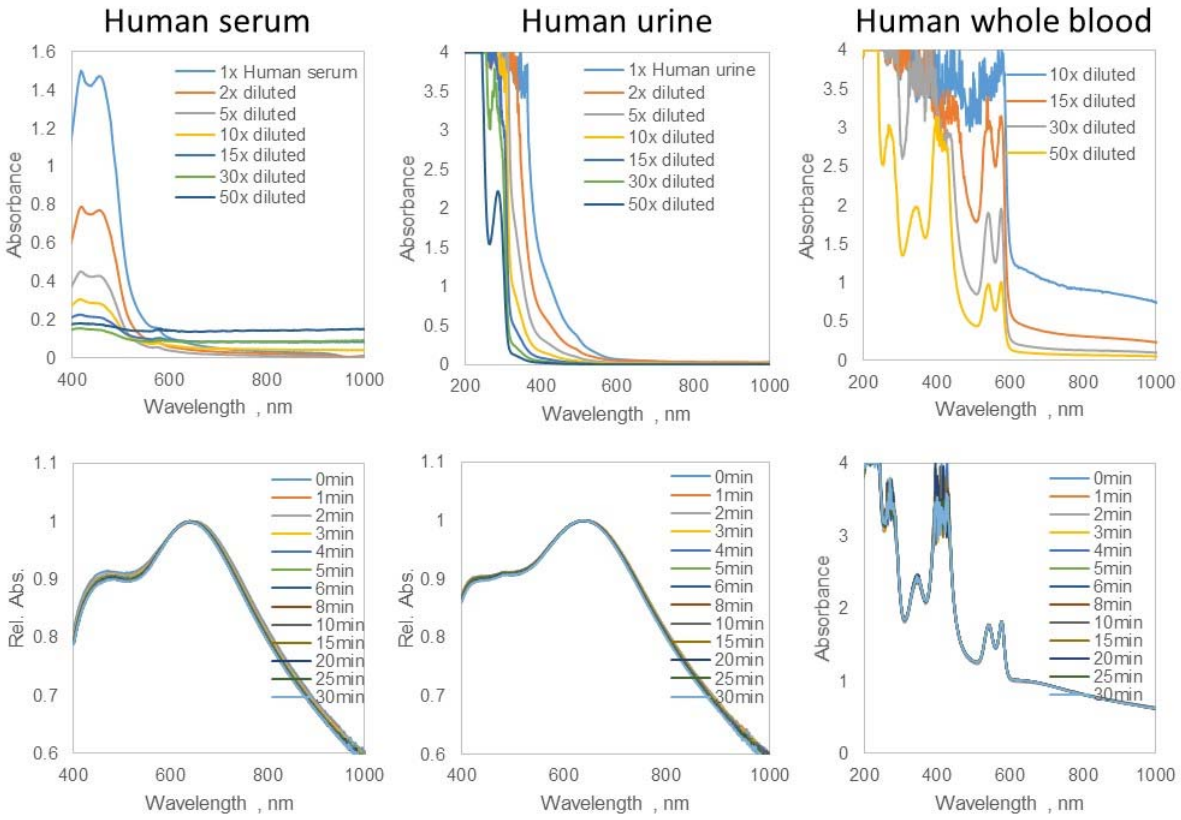

Fig. S1 Charaterization of absorption spectra and colloidal stabilty of PVP-AuAg in body fluids. No significant absorption was observed in the range of >600 nm in all case of human serum, urine and whole blood with 1:50 dilution (top lane). Colloidal stability was measured by UV-Vis spectrophotometer upto 30 min after the mixing of PVP-AuAg and body fluids. There was no decrese of absorption intensity in all the samples indicating that colloidal stability of the particles was well maintained.