Fabrication of Hollow Platinum Nanospheres as Electrocatalyst for Conductometric Immunoassay of Interleukin-6 in Human Serum

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This letter describes the preparation of hollow platinum (Pt) nanospheres as electrocatalyst for conductometric immunoassay of interleukin-6 (IL-6), as a model protein, in human serum.

Metal nanostructures attract considerable attention scientifically as well as industrially, because they have possible uses in diverse applications such as catalysis, devices, transistors, and optoelectronics.[1] Solid platinum (Pt) nanoparticles were found displaying strong catalytic activity on bioelectrocatalytic reaction.[2] Some methods based on nanoplatinum probes have been developed for DNA detection or immunoassay. However, a critical problem with Pt-based catalysts is their prohibitive cost. Thus, economical and effective alternative catalysts are required, and cost-effective routes are being sought to make more-efficient Pt catalysts. To date, great efforts have been made worldwide to develop and improve Pt catalysts with a high surface area to achieve high catalytic performance and utilization efficiency.

Recently, nanomaterials having hollow interiors have attracted increasing interest because of their specific structures, interesting properties, that differ from their solid counterparts, and wide applications in chemistry, biotechnology, and materials science.[3] The electrocatalytic properties of hollow nanosphere labels could be used for signal amplification. Hyeon and co-workers demonstrated that hollow Pd spheres exhibited good catalytic activities in Suzuki cross-coupling reactions and could be reused many times without the loss of catalytic activity.[4] Liang and co-authors fabricated 24-nm hollow Pt nanospheres, which displayed good electrocatalytic activity in the oxidation of methanol.[5] To the best of our knowledge, however, there is litter report focusing on conductometric immunoassay for the study of the antigen–antibody interaction by using hollow Pt nanospheres as labels.

Herein we prepared specially shaped hollow Pt nanospheres with an average diameter of 20 nm and found their catalytic efficiency to be 100-fold greater than that of solid Pt nanospheres. Using the hollow Pt nanosphere-modified anti-IL-6 as in situ amplified probes, we established a simple and sensitive sandwich-type conductometric immunoassay method for the detection of IL-6 in human serum in 0.02 M phosphate buffer solution (pH 7.0) containing 50 μM H_2O_2, 0.01 M KI, and 0.15 M NaCl (Figure 1a). The principle of the detection is based on the changes of the conductivity between two parallel electrodes by many biochemical reactions in solution before and after the antigen–antibody interaction.

At the first step, aqueous silver colloids (50 mL, silver atomic concentration of 1.0 mM) with an average diameter of 16 ± 1.0 nm were prepared by reducing $AgNO_3$ with sodium citrate tribasic dehybrate.[6] Secondly, porous hollow Pt nanospheres (20 nm in diameter) were prepared consulting the literature.[7] Briefly, 10 mL of a 0.26 M aqueous solution of H_2PtCl_6 and 6 mL of a 0.1 M aqueous solution of L-ascorbic acid were added into 34 mL of the above-prepared silver colloidal solution and continuously stirred for another 20 min to yield nanoparticles with Ag–Pt alloy shells. In the chemical etching treatment of newly prepared nanoshells, 50 mL of (1 M) NH_4OH solution was added to the as-prepared Ag–Pt alloy hollow spheres solution. The reaction mixture was centrifuged at 15000 rpm for 30 min to remove the Cl ions. Afterwords, 50 mL (1.0 M) of HNO_3 was employed to remove silver atoms from the Ag–Pt alloy shells. This centrifugation procedure needed to be repeated several times. Vigorous magnetic stirring was maintained throughout the synthesis. Figure 1b shows the TEM image of the as-prepared porous hollow Pt nanospheres.

Protein could be attached in the pores of porous materials by simply mixing the porous hollow Pt nanospheres with the protein solution. To further monitor the formation of the porous hollow Pt nanospheres, we used N_2 adsorption–desorption isotherm to measure the specific surface area and pore size distribution of powdered or solid materials. In the range of 0.7–1.0 Pa, step-like curves were due to capillary condensation taking place in porous material. BET surface area was 746.3 m^2/g according to the BET equation (Figure 2). Moreover, the narrow pore size distribution curve showed that pore size with BJH diameter of the most proble distribution was 1.8 nm (data not shown).

To investigate the performance of the porous hollow Pt nanospheres, the as-prepared nanospheres were employed as immobilized affinity supports for anti-IL-6 conjugation via direct adsorption of anti-IL-6 antibodies on the surface of porous hollow Pt nanospheres in conductometric immunoassay. The immunoassay format was employed to detect IL-6 in solution since the catalytic reaction of the carried Pt nanoparticles

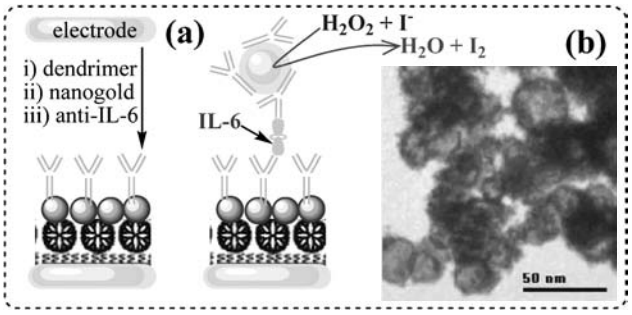

Figure 1. (a) Schematic representation of the preparation of an immunosensing layer and schematic view of conductometric detection of IL-6, and (b) SEM image of the porous hollow Pt nanospheres.

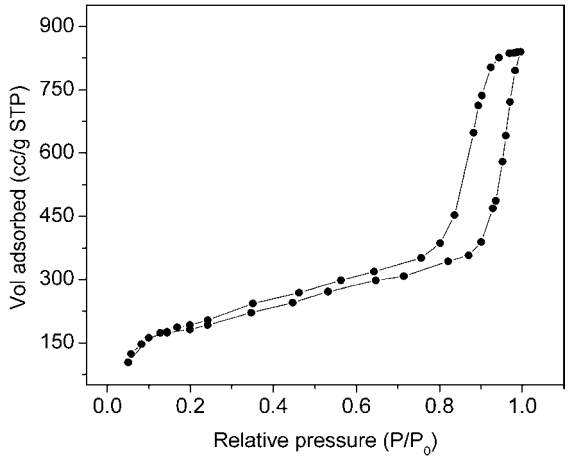

Figure 2. Nitrogen adsorption–desorption isotherms at 77 K for the calcined porous hollow Pt nanospheres.

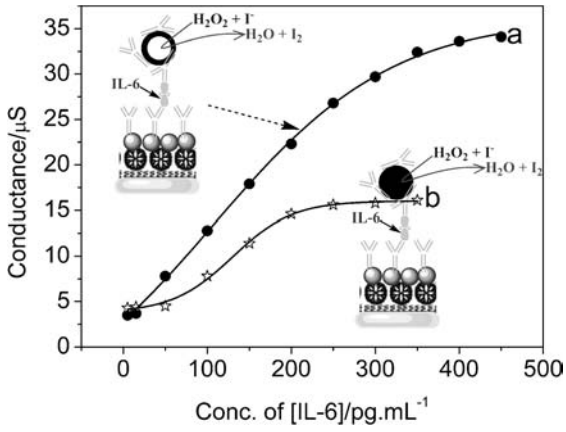

Figure 3. Calibration curves for the conductometric sandwich-type immunoassay of IL-6 recorded using (a) porous hollow Pt nanospheres, and (b) solid Pt nanospheres as labels.

was accompanied by changes in the free iodine concentration when KI is used as a supporting electrolyte. For the preparation of a sandwich-type conductometric immunosensor, an anti-IL-6 layer was constructed on a dendrimer G4 and colloidal gold-modified interdigitated microelectrode according to our report previously (Figure 1a).[8]

First, we investigated the electrocatalytic response of the fabricated hollow Pt nanospheres in 0.02 M PBS (pH 7.0) containing 50 μM H_2O_2, 0.01 M KI, and 0.15 M NaCl on the basis of the change in conductivity before and after the antigen–antibody reaction. The measurement method is as follows: 30 μL of serum sample was added into the 70 μL of incubation solution (pH 7.0, PBS), and then the as-prepared immunosensor was incubated in the incubation solution at 35 °C for 20 min. After a washing step with doubly distilled water, the resulting substrates were submerged in hollow Pt nanosphere-labeled anti-IL-6 solution for 20 min at 35 °C. After rinsing thoroughly with pH 7.0 PBS to remove the unbound nanocomposites, conductometric measurement were carried out on the catalytic reduction by the hollow Pt nanospheres in 0.02 M PBS (pH 7.0) containing 50 μM H_2O_2, 0.01 M KI, and 0.15 M NaCl towards KI and H_2O_2. As shown in Figure 3a, a progressive increase in the conductivity would be obtained with increasing the IL-6 concentration in incubation solution. The data indicates that the immunosensor is capable of distinguishing IL-6 concentration range from 15 to 350 pg/mL with a relatively low detection limit of 8 pg/mL at a signal-to-noise ratio of 3. Compared with our previous report,[8] the linear range and detection limit were obviously improved.

For comparison, we also used the solid Pt nanosphere (20 nm in diameter)-labeled anti-IL-6 as secondary antibodies for the detection of IL-6 following the same protocols, and the linear ranges and detection limits are 50–200 pg/mL with a detection limit of 20 pg/mL IL-6 (Figure 3b). It revealed that the synthesized hollow Pt nanospheres-labeled protocol possessed the advantages of more sensitivity and wider linear range compared with those of directly using solid Pt nanospheres as labels. The reason may be the fact that the hollow Pt nanospheres could exhibit larger surface area than that of solid Pt nanospheres, which enchanced the catalytic effectivity of Pt nanospheres.

In summary, we described a simple and sensitive conductometric immunoassay method by using hollow Pt nanospheres as nanocatalyst-labels. Although the present assay system is focused on the determination of the target antigen molecules, it can be easily extend to the detection of other antigens or biocompounds. Further investigations are going on to develop a microarray structure for our functional nanocatalyst-label-based protein assay and to test them ultimate sensitivity.

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