Efficient electrocatalytic oxidation of NADH at gold nanoparticles self-assembled on three-dimensional sol-gel network

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It is demonstrated that gold (Au) nanoparticles self-assembled on a sol-gel derived three-dimensional silicate network efficiently catalyze the oxidation of NADH in the absence of any electron transfer mediators with a decrease in overpotential of 780 mV in neutral solution.

The electrochemical oxidation of NADH in aqueous solution has attracted considerable interest in order to develop amperometric biosensors for the detection of biomolecules which react under enzymatic conditions with NAD^+ to produce NADH.[1] Although the formal potential of the $NADH/NAD^+$ couple in neutral pH at 25 °C is estimated to be −0.56 *vs.* SCE, significant overpotential as large as 1.0 V is often required for the direct oxidation of NADH at bare electrodes.[2] The large overpotential required for the oxidation invites interference from other easily oxidizable species present in the real samples. In addition, the direct oxidation at bare electrodes is often accompanied by the fouling of the electrode surface by the adsorption of reaction intermediates. A way to decrease the high overpotential and avoid the fouling effect is to use a suitable electrocatalyst that can facilitate the electron transfer kinetics. In this way, much effort has been made to identify new materials which can effectively overcome the kinetic barriers for the electrochemical regeneration of NAD^+.[3]

Metal nanoparticles are increasingly used in many electrochemical, electroanalytical and bioelectronic applications owing to their extraordinary electrocatalytic activity.[4–8] Willner's group explored the possible utilization of Au nanoparticles for biosensing, bioelectronic and optical applications.[9] The catalytic activity of these nanosized particles was linked to a band gap of a metallic–insulator transition in the few nanometer range, high surface area and interface-dominated properties that differ from those of the bulk counter-parts.[4–6] As a part of our continuing effort to develop an efficient electrocatalyst for the oxidation of NADH, we have exploited nanosized Au particles as a catalyst for the oxidation of NADH. Herein, we report the proof-of-concept demonstration of the electrocatalytic activity of nanosized Au particles immobilized on the thiol tail groups of the sol-gel derived 3-D silicate network towards the oxidation of NADH.

Scheme 1 displays the schematic representation for the self-assembling of Au nanoparticles (*nanoAu*) on (3-mercaptopropyl)-trimethoxysilane (MPTS) modified polycrystalline gold (polygold) electrode (area: 0.031 cm^2). The MPTS sol was prepared by dissolving MPTS in methanol/water/0.1 M HCl mixture in the molar ratio of 1 : 3 : 3 and stirring the mixture vigorously for 30 min. The Au nanoparticles of diameter 2.6 nm were prepared

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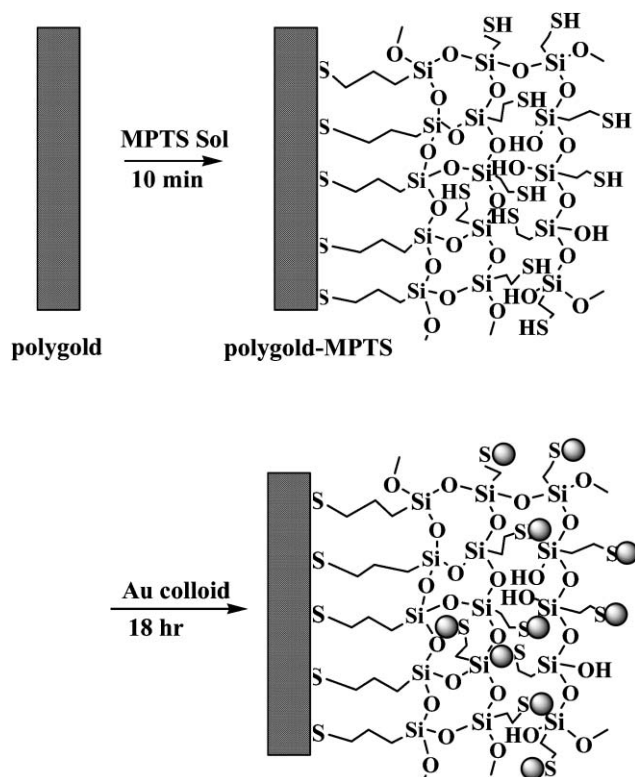
Scheme 1 Schematic representation of the self-assembling of Au nanoparticles on polygold–MPTS electrode.

by the usual procedure.[10] The electrochemically cleaned polygold electrode was first modified with the self-assembly of MPTS by immersing it in the MPTS sol for 10 min. The resulting polygold–MPTS electrode was then rinsed well with water and soaked in colloidal *nanoAu* solution for 18 h. The *nanoAu* particles chemisorb onto the thiol groups by covalent bonding. These particles are distributed throughout the silicate network (Scheme 1).

Fig. 1 shows typical cyclic voltammograms of NADH on polygold–MPTS and *nanoAu* self-assembled polygold–MPTS electrodes in 0.1 M phosphate buffer solution (pH 7.2). The bare polygold electrode and polygold–MPTS electrodes did not show any characteristic voltammogram for the oxidation of NADH in the potential range used in the investigation. The polygold electrode shows an ill-defined voltammogram at > 850 mV and the oxidation peak is shifted to more positive potential in the subsequent cycles, indicating that the electrode surface undergoes fouling by oxidation products. Interestingly, as shown in Fig. 1(d), the *nanoAu* self-assembled polygold–MPTS electrode shows an
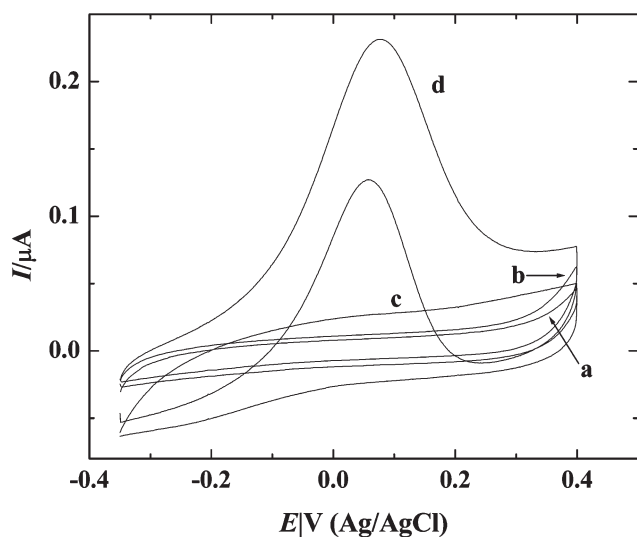
Fig. 1 Cyclic voltammograms of polygold–MPTS (a, b) and *nanoAu* self-assembled polygold–MPTS (c, d) electrodes in the absence (a, c) and presence (b, d) of 0.5 mM NADH in 0.1 M phosphate buffer (pH 7.2). Scan rate 10 mV s^{-1}.

inverted V shape voltammogram for the oxidation of NADH at 0.07 V. A 780 mV decrease in the overpotential concomitant with a significant increase in the peak current has been observed at this electrode. The special inverted V shape of the voltammogram implies that the oxidation process involves surface bound species. Such a characteristic voltammetric response was not observed in our experiment at the polygold electrode. The voltammogram is very stable and the peak position and peak current remained unchanged in the subsequent sweeps, showing that the *nanoAu* self-assembled electrode does not undergo fouling by the oxidation products. It is worth mentioning here that the onset potential for the oxidation of NADH is more negative ($\sim$ −0.3 V), indicating the excellent catalytic activity of *nanoAu* particles. The voltammetric peak for the oxidation of NADH increases linearly with increase in the concentration of NADH. Fig. 2 displays the voltammograms for NADH at *nanoAu* self-assembled polygold–MPTS electrode at different scan rates. The peak potential slightly shifts to positive potential as the scan rate is increased. The i_p^a *vs. $v^{1/2}$* plot is approximately linear at lower scan rate (Fig. 2B), indicative of a diffusion controlled process of NADH oxidation. The *nanoAu* particles on the sol-gel network act like a nanoscale electrode and provide a conduction pathway. Since the *nanoAu* particles are distributed both inside the sol-gel network and on the electrode surface, the active sites of *nanoAu* particles effectively partake in the catalytic oxidation process. The catalytic effect of nanosized gold particles can be rationalized by considering the incipient hydrous oxide/adatom mediator model.[11–13] The *nanoAu* surface undergoes oxidation at unusually low potential, resulting in the formation of incipient oxides.[12] These surface oxides apparently exist at the interface either as AuOH or Au(OH)$_3$ and can mediate the oxidation of substrate in solution.[10,13] In the present case, NADH forms a complex with the surface bound oxides as is observed for other substrates; this mediator–NADH complex undergoes further reaction to yield NAD$^+$ and the surface bound oxide mediator. The electrocatalytic features at the *nanoAu* self-assembled electrode resemble those observed at a gold single
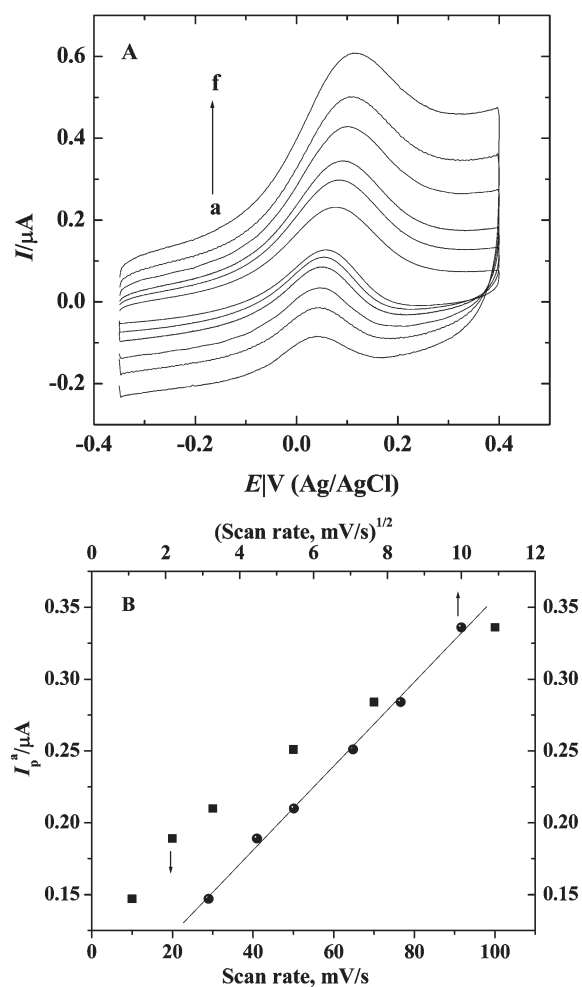
Fig. 2 (A) Cyclic voltammograms of NADH (0.5 mM) at *nanoAu* self-assembled polygold–MPTS electrode in 0.1 M phosphate buffer (pH 7.2). Scan rate: (a) 10, (b) 20, (c) 30, (d) 50, (e) 70 and (f) 100 mV s^{-1}. (B) Corresponding plots of peak current against scan rate and square root of scan rate.

crystal electrode, Au(111), for the oxidation of NADH.[14] The electrocatalytic effect of Au(111) electrode was associated with the partially discharged surface bound oxides, which function as a strong mediator in neutral pH.

The electrocatalytic activity of the *nanoAu* self-assembled polygold–MPTS electrode is remarkably stable against repetitive cycling in the potential window used. As the present electrode shows stable electrocatalytic response with a significant decrease in the overpotential without any additional redox mediator, it could find application in the field of electrochemical biosensors. The present result is, to the best of our knowledge, the first example that demonstrates the electrocatalytic effect of nanosized gold particles towards the oxidation of NADH. The electrocatalytic activity of nanosized Au particles toward the oxidation of NADH is attributed to the function of surface bound oxides as an efficient oxidation mediator. Detailed studies on the observed electrocatalytic effect including the mechanism of the oxidation reaction, particle size dependency, the effect of substrate used for the self-assembling of *nanoAu* and the utilization of this NADH transducer for sensing applications are underway.

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