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A New Method for the Synthesis of Selenium Nanoparticles and the Application to Construction of H_2O_2 Biosensor

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Abstract: The well-distributed, stable selenium nanoparticles (10 nm) with good adhesive ability and biocompatibility were successfully synthesized by using the template of chitosan cross-linked with glutaradehyde. The resulting selenium nanoparticles were used as a new carrier for horseradish peroxidase to construct H_2O_2 biosensors with good performances.

Keywords: Selenium nanoparticle, synthesis, immobilization, H_2O_2 biosensor.

To date the most popular biosensor reported in the literature are those employing redox enzymes coupled with amperometric detection. For the construction of this kind of biosensors, a general, broadly applicable method for enzyme immobilization still needs to be discovered. One of recent trends is based on the use of new carriers, such as nanosized particles[1,2].

Amorphous selenium nanoparticles (SN) are demonstrated not only unique photoelectric, semiconducting and X-ray-sensing properties, but also biological activity and good adsorptive ability due to interaction between the nanoparticles and NH, C=O, COO^-, and C-N groups of the proteins[3-5]. So they may be used as new carriers for the constitution of redox enzymes based biosensors.

In order to realize this purpose, firstly, we are interested in fabricating stable selenium nanoparticles with good adhesive ability and biocompatibility. Generally, stable SN can be synthesized in polymer media such as hydroxyethylcellulose[6], poly(vinylpyrrolidone)[7] and poly(2-acrylamido-2-methylpropane sulfonic acid)8 *etc.*. In this paper, natural biopolymer chitosan cross-linked with glutaraldehyde was selected as a template to synthesize SN because of its unusual property of combination, including excellent membrane-forming ability, high permeability towards water, good adhesion, biocompatibility and high mechanical strength. Then the resulting selenium nanoparticles were coated on glassy carbon electrode and form a stable and even film. Finally, we immobilized horseradish peroxidase (HRP) onto the selenium nanoparticle-layer to develop the H_2O_2 biosensor. Because of the biocompatibility of selenium and chitosan, the nanoparticle-layer can supply a biological environment for the

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enzyme to retain its native structure and activity. Thus, the electrochemical biosensors as-obtained could exhibit fine performance.

The reaction of chitosan cross-linked with glutaraldehyde was initiated by mixing of 50 μL of 0.10% chitosan solution and 50 μL of 1.0% glutaraldehyde solution. Then, 25 μL of 0.5 mol/L selenious acid was added. Finally, 25 μL of 1.0 mol/L ascorbic acid was added to the mixture solution under shaking. In the chitosan network structure, selenious acid was reduced to elemental selenium that could grow into SN in control. The morphology of SN obtained was measured by TEM (**Figure 1**).

The sensor was prepared by spreading 5 μL SN colloid on the surface of electrode. The compact selenium nanoparticles layer (CSNL) with a uniform structure was formed on the surface of electrode after storage in the refrigerator at 4℃ overnight (**Figure 2**). Then, 5 μL of the HRP (pH 7.8, 2 mg/mL) was deposited on the surface of CSNL, and the electrode was stored at 4℃ in refrigerator again. When HRP solution was dropped on the CSNL, the SN can combine with HRP molecules very well except slight fold (**Figure 3**). Contrasting the two SEM pictures (**Figure 2** and **3)**, it can be seen that the size of particles/HRP is bigger than that of SN alone. The information suggested that the SN had adsorbed the enzyme molecules.

Electrochemical measurements were performed on a CHI 630A electrochemical analyzer with a three-electrode system including a platinum wire as auxiliary electrode, a saturated calomel electrode (SCE) as reference electrode, and the H_2O_2 sensor as working electrode.

Figure 4 showed the cyclic voltammograms of the sensor in different H_2O_2 concentration solution. When H_2O_2 was added, the obvious catalytic characteristic appeared with a dramatic increase of the reduction currents and a sharp decrease of the oxidation currents in the presence of 2.0 mmol/L catechol. The change in redox currents displays an obvious electrocatalytic behavior of immobilized HRP to the reduction of H_2O_2 Furthermore, the reduction peak currents increased with increasing H_2O_2 concentration.

Figure 1 TEM of selenium nanoparticles synthesized in the chitosan matrix (magnification 50 000).

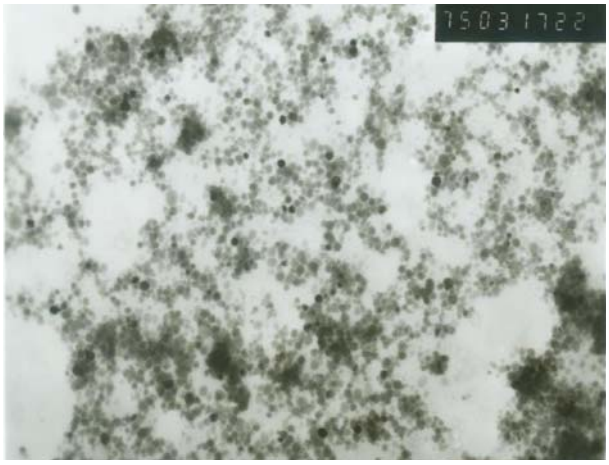


Figure 2 SEM of selenium nanoparticles on the surface of glassy carbon electrode.

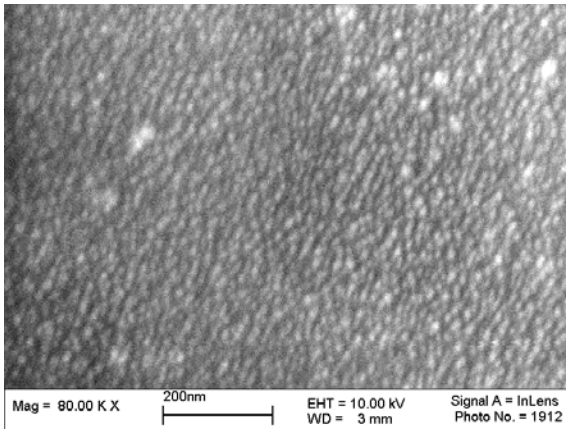


Figure 3 SEM of selenium nanoparticles /HRP on the surface of glassy carbon electrode

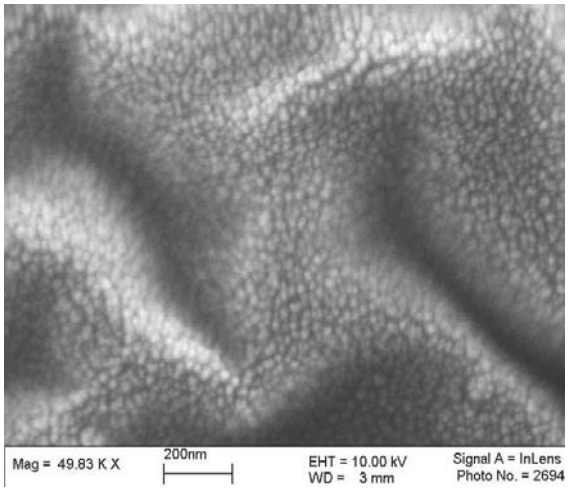
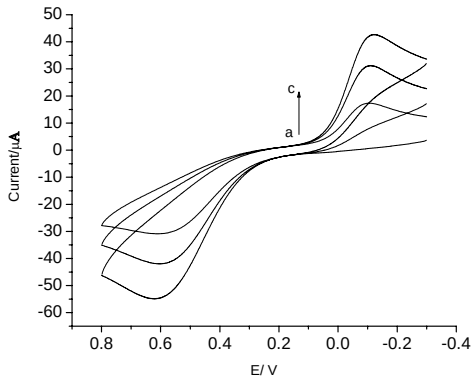


Figure 4 Cyclic voltammograms of the sensor in pH 7.0 PB solution at 100mV/s.



H_2O_2 concentration: 0.00, 106 and 211 μmol/L H_2O_2 (from a to c).

Both redox peak currents and peak-to-peak separation increased with increasing scan rate. There is linear relation between the redox peak currents and square root of scan rate. The effect of solution pH on the reduction currents of catechol exhibited a maximum response in the pH 7.0 phosphate buffer solution.

The steady-state response of this sensor to H_2O_2 was measured at potential of –120 mV. It achieved 95% of steady-state currents within 10 s. With increasing H_2O_2 concentration the amperometric response of the sensor increased. The linear response range of the sensor to H_2O_2 concentration is from 1.7×10^{-6} to 5.3×10^{-4} mol/L with a correlation coefficient of 0.998 (n=12) and a detection limit of 9.2×10^{-7} mol/L at a signal-to-noise ratio of 3.

The apparent Michaelis-Menten constant is determined to be 0.31 mmol/L. The value is lower than 4.8 mmol/L for the silica sol-gel entrapped HRP[9] and 9.5 mmol/L for the silk fibroin membrane-entrapped HRP[10]. These results indicate that HRP molecules immobilized on SN exhibit higher affinity to H_2O_2 .

The storage stability of the sensor was examined by amperometric response at H_2O_2 concentraation of 106 μmol/L. After 60 days of storage and intermittently measuring every five days, the results showed that the response of the sensor still maintain about 80% of the original values.

In summery, the selenium nanoparticles synthesized in chitosan is to be a promising carrier for the preparation of practical biosensors.

Acknowledgment

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