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Pt-Au bimetallic nanoparticles with peroxidase-like activity and their applications for ascorbic acid(AA) detection

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Abstract

Nano-enzymes are the material which can catalyze the substrate reaction of natural enzymes, and have a catalytic mechanism to that of natural enzymes. The raise of nano-enzymes provides a new way for medical treatment on the identification of cancer cells. For an instance, coupling the magnetic nano-particales with antibodies can specifically identify the cancer cells. Even, the free radicals provided by the tiny amounts of magnetic nano-particles can also kill cancer cell..

Keywords

Au@Pt nanoparticles, nano-enzymes, ascorbic acid, PH.

1. Introduction

Ascorbic acid (AA), also called Vitamin C, the two adjacent enol hydroxyl groups in the second and third positions of the molecule are easy to dissociate and release H+, so they have the properties of acids. Due to the strong reducibility of the enediol groups in the molecular structure, the enediol groups are easy to be oxidized to diketone groups and become dehydroascorbic acid. In a strong base solution, the lactone ring can be hydrolyzed to form ketoates. The solubility of AA in water is 0.3 g/mL, the melting point is 190 to 192 ℃, the redox potential is E=0.166V when the pH= 4, and its ionization constant PK1= 4.17, PK2= 11.57. Vitamin C molecule has conjugated double bonds, its diluted hydrochloric acid solution has the maximum absorption at 243nm. Under neutral or alkaline conditions, the redshift reaches 265nm.

AA can promote the synthesis of collagen and the formation of tetrahydrofolic acid. Since AA always participate in a complex redox reaction, it is often used in antioxidants, nutritional fortifier added to food and drugs. When the human body lacks AA, scurvy and decline in human immunity may occur.

AA is a necessary nutrient provided by the outside world. In recent years, a series of detection methods have been developed, such as (1Titration method (2 spectrophotometry (3 electrochemical methods (4 chromatography.

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Those methods above have the advantages of simplicity, rapidity and reliability. However, due to the high overpotential of AA and easy contamination of the electrode on the ordinary electrode, the detection reproducibility is very poor. These shortcomings make it difficult for the bare electrode to directly detect AA in the sample.

In our study, Au@Pt bimetallic nanoparticles with peroxidase-like activity are prepared. They have no surfactant and more exposed catalytic sites, porous structure with a large specific surface area. Also, gold and platinum have a synergistic effect. A detection method is proposed in this research by utilizing the strong reducibility of AA and the peroxidase-like activity of Au@Pt nanoparticles.

2. Results

2.1 Characterizations of the Au@Pt nanoparticles

In synthesizing the Au@Pt nanoparticles, $HAuCl_4$ and H_2PtCl_6 are used as the metal precursors of Au and Pt respectively. Firstly, sodium citrate is used as a reductant to produce Au nanoparticles in the boiling $HAuCl_4$ solution. Next, $NaBH_4$ is used to reduce H_2PtCl_6 to produce Pt atoms combined with Au atoms, thus producing the Au-Pt alloy. A brief schematic illustration is displayed in Fig. 1 and the detailed processes are stated in the Experimental section. The method used to synthesize the Au@Pt nanoparticles in this research is different from that more commonly used in others' works. In the works done by Chenghui Tan et.al.[1], Min Wei et.al.[2] and Fanjun Zhu et.al.[3], ascorbic acid is used as the reductant to produce Pt from its precursor H_2PtCl_6, while in our work $NaBH_4$, a reductant with relatively feeble reducibility, is chosen to supplant ascorbic acid. Considering the strong reducibility of ascorbic acid, the reaction would be too fast to produce Pt atoms evenly distributed on the surfaces of the Au particles, while the reaction slows down to meet the demand by replacing ascorbic acid with $NaBH_4$.

The as-prepared Au@Pt nanoparticles are first characterized by Transmission Electron Microscope (TEM) image (Fig. 2) in which the particles are found to be nearly round with their diameters less than 20 nm.

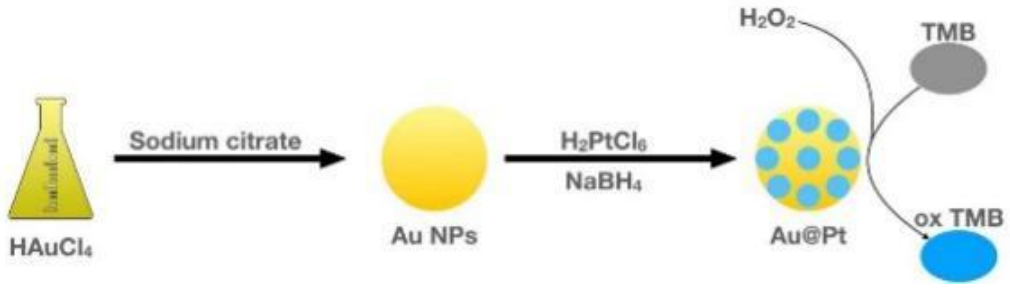

Fig. 1 Schematic illustration of Au@Pt synthesis

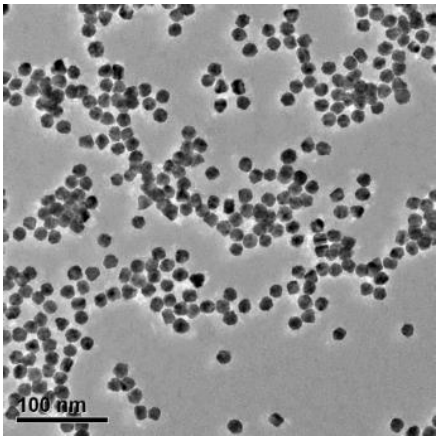

Fig. 2 TEM image of Au@Pt nanoparticles

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2.2 The peroxidase-like activity of the Au@Pt nanoparticles

The as-prepared Au@Pt nanoparticles are found to have a peroxidase-like activity. In the presence of H_2O_2, 3, 3′, 5, 5′-tetramethylbenzidine (TMB) can be catalyzed to give out a colorimetric reaction in which the substrate turns blue. Since this oxidation reaction features the blue color whose maximum absorbance is at 652 nm, the absorbance at 652 nm is used to measure the reaction activity in experiments of this research. To further investigate the peroxidase-like activity of the Au@Pt nanoparticles, experiments are designed to figure out the optimal reaction conditions. Factors affecting reactions catalyzed by the Au@Pt nanoparticles with TMB and H_2O_2 as the substrates are shown in Fig. 3.

As is shown in Fig. 3, the optimal pH for the reaction catalyzed by Au@Pt nanoparticles is 4 (Fig. 3a).

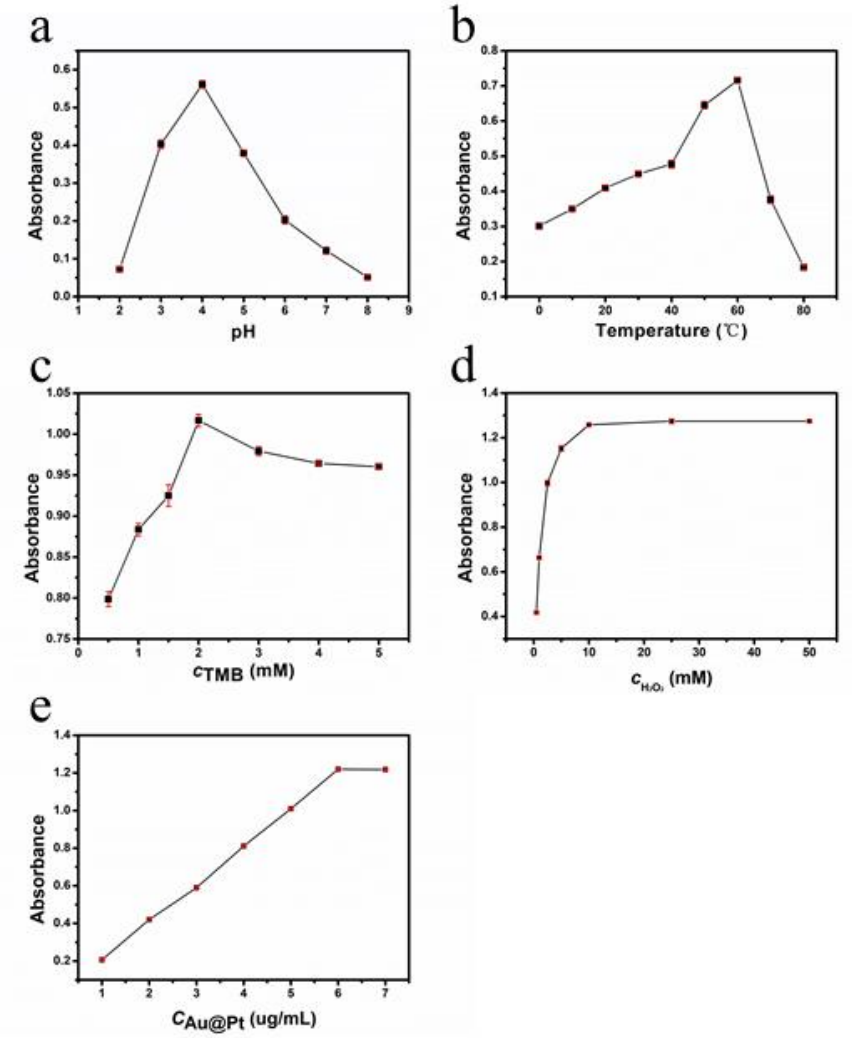

Fig. 3 Dependance of the absorbance at 652 nm on pH(a), temperature (b), concentration of TMB(c), concentration of H2O2(d) and concentration of Au@Pt(e)

When the reaction is carried out under 60 ℃ the nanoparticles show the highest activity (Fig. 3b). Also, the reaction carried out in the optimal pH and temperature conditions (pH=4, Temperature=60 ℃) demonstrates the activity to be dependent on concentrations of the substrates. In Fig.3d, the reaction activity increases as the concentration of H_2O_2 increases until about 10 mM. While in Fig.3c, as the concentration of TMB increases, the reaction activity first increases and then starts to descend at about 2 mM. In explaining this phenomenon, we observed that since TMB is insoluble in water, excessively high concentration of TMB leads to precipitation of this insoluble substance on the surfaces of nanoparticles, covering the combining site of the nanoparticles for the

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substrates and thus halting the process of the reaction. The influence of the amount of the Au@Pt nanoparticles is also examined (Fig. 3e) and the reaction activity is found to be positively correlated to the concentration of the Au@Pt nanoparticles.

2.3 Detection of ascorbic acid (AA)

A detection method of ascorbic acid is carried out based on the intrinsic peroxidase-like activity of the Au@Pt nanoparticles. Detection methods using nanomaterials as enzyme mimetics have been reported in many other works. For instance, glucose generates H_2O_2 when catalyzed by glucose oxidase (GOx), which is utilized in the detection of glucose[4]. In this method, in the presence of H_2O_2, the nanomaterial catalyzes a colorimetric reaction which indicates the amount of glucose indirectly since H_2O_2 is generated by the oxidation of glucose.

Different from glucose, oxidation of AA does not produce H_2O_2. However, with strong reducibility, AA can consume H2O2 rapidly, which can be utilized to design a detection method where the rate of the colorimetric reaction is negatively correlated to the amount of AA. (Fig. 4)

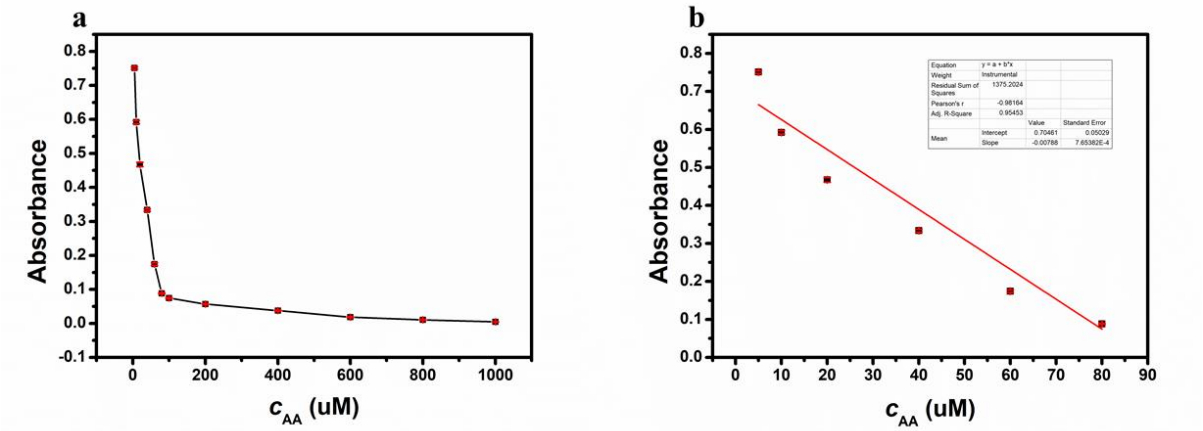

Fig. 4 Dependance of the absorbance at 652 nm on the concentration of AA(a) and the linear calibration plot(b)

The dependence of the absorbance at 652 nm on the concentration of AA is shown in Fig. 4a. The intensity of the absorbance at 652 nm goes down as the concentration of AA increases, which is in line with our prediction. The linear calibration plot is shown in Fig. 4b, which gives out the linear range for AA detection as from 5 μM to 80 μM and the limit of detection (LOD) as 5 μM.

3. Experimental section

3.1 Preparation of the Au@Pt

The method we used to prepare the Au@Pt is similar to that used by Fanjun Zhu et.al.. In a typical synthesis, 10 mL 1mM $HAuCl_4$ is heated with stirring to boiling. Then, 1 mL 38.8 mM sodium citrate is quickly added to the vortex of the solution, turning the color into purplish red from the previous light yellow. Keep the solution boiling for 10 more minutes and then stop heating but keep stirring the solution to cool it down to the room temperature. The solution is then centrifuged and washed three times by deionized water. Next, diffuse the substance into 8 mL deionized water. Heat the solution to 80 ℃ with stirring and quickly add 1 mL 10 mM H_2PtCl_6 as well as 1 mL 10mM $NaBH_4$ into the solution. Stir the solution for 10 minutes at 80 ℃ and then stop heating but keep stirring the solution to room temperature. At last, the solution is centrifuged, washed by deionized water three times and the particles are diffused into 5 mL deionized water.

3.2 Optimal reaction conditions

In investigating the optimal pH condition for the reaction, PBS of seven different pH values (pH=2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0) were prepared as well as 20 mM TMB dissolved in DMSO and 50 mM H_2O_2 dissolved in water. In the experiments, 0.6 mL PBS (pH ranges from 2.0 to 8.0), 10 μL TMB,

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10 μL H_2O_2 and 20 μL nanoparticles were put together. After 10 minutes of reaction, the absorbances at 652 nm of the solution were examined and recorded. Each group had two repetitions.

To figure out the optimal temperature for the reaction, nine groups of 0.6 mL PBS (pH=4.0) were set under different temperatures (0, 10, 20, 30, 40, 50, 60, 70, 80 ℃) for 8 minutes. 20 μL nanoparticles, 10 μL 50 mM TMB, and 10 μL 50 mM H2O2 were then added to the solution of each of the nine groups which were still kept in their respective temperature. After 10 minutes of reaction, the absorbances at 652 nm were examined and recorded. Each group of the experiments had two repetitions.

3.3 Influence of the concentrations of the substrates and the catalyst

Seven groups of 0.6 mL PBS (pH=4.0) were set at 60 ℃ for 8 minutes and then 20 μL nanoparticles were added into each group. 0.5, 1, 2.5, 5, 10, 25, 50 mM H_2O_2 (ultimate concentration) were subsequently added to each group, which was followed by adding 10 μL TMB. 10 minutes later the absorbances at 652 nm were examined and recorded. Each group had two repetitions.

Similarly, seven groups of PBS were prepared in identical conditions with the previous experiment. 20 μL nanoparticles were added to the solutions and then 0.5, 1, 1.5, 2, 3, 4, 5 mM TMB (ultimate concentration) were added to the solutions. Next, 10 μL H_2O_2 was added to each of the groups. 10 minutes later the absorbances at 652 nm were examined and recorded. Each group had two repetitions.

In addition to the substrates, the influence of the amount of the catalyst was also investigated. Seven groups of PBS were prepared in the same condition, 10 μL 2 mM TMB and 10 μL 50 mM H_2O_2 were added to the solutions. Different concentrations (1, 2, 3, 4, 5, 6, 7 μg/mL) of the nanoparticles were added into each of the groups. After 10 minutes of reaction, absorbances at 652 nm were examined and recorded. Each group had two repetitions.

3.4 Detection of AA

Twelve groups of PBS were prepared in the same condition, 10 μL 2 mM TMB and 10 μL 50 mM H_2O_2 were added to the solutions. 5, 10, 20, 40, 60, 80, 100, 200, 400, 600, 800, 1000 μM AA were added to the solutions and after 10 minutes of reaction absorbances at 652 nm were examined and recorded. Each group had 2 repetitions. Notably, since the reducibility of AA is much stronger than that of TMB, AA is always consumed first so there is no need to carry out the two reactions separately.

4. Conclusion

In this research, we prepared the Au@Pt particles by using sodium citrate and $NaBH_4$ as the reductants. The optimal conditions for the catalyst were figured out to be pH=4.0, temperature=60 ℃. Besides, the influences of the concentrations of substrates and the catalyst were investigated. A method for detecting ascorbic acid was developed in which the LOD of ascorbic acid was down to 5 μM and the linear range was from 5 to 80 μM. Our work provides a new avenue for detection methods based on the peroxidase-like activity of nanoparticles. Besides, the method to detect AA may contribute to other works associated with AA.

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