Synthesis of Au@Pt nanoparticles for detection of ascorbic acid

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Abstract: Nanoenzyme is a kind of mimic enzyme with unique properties of nanomaterials and catalytic function. They have been developed to address the limitations of natural enzymes and conventional artificial enzymes. Compared with traditional mimic enzymes, nanoenzymes have the advantages of higher catalytic efficiency, low costs, high stability against stringent reaction conditions. The catalytic reaction of nanoenzymes is the same as natural enzymes, which depends on pH, temperature and substrate concentration. Taking advantage of the high catalytic properties of bimetallic alloy nanoparticles, we prepare Au@Pt nanoparticles with high catalytic activity and use it to detect ascorbic acid. As a mimic oxidase, Au@Pt nanoparticles shows the good biocompatibility of Au@Pt nanoparticles for promising bioapplications. [1]

Keywords: Au@Pt nanoparticles, detection of ascorbic acid, nanoenzymes

1. Introduction

Nanoenzymes are divided into noble metal nanoenzymes, metal oxide nanoenzymes and carbon-based nanoenzymes. Nanomaterials have a variety of oxidoreductase activities, including peroxidase (POD), catalase(CAT), superoxide dismutase(SOD), oxidase(OXD), etc.

Due to the unique physical and chemical properties of precious metal materials, noble metal nanomaterials have become an important part of nanomaterials and have been widely used in electrical, optical, magnetic and mechanical fields. In recent years, their applications in the field of sensing have reached a new level. The using of noble metal nanomaterials not only makes the original sensing technology possess some of its own characteristics, but also greatly improves some performance parameters in the measurement process, such as sensitivity and detection time. And because of the noble metal particles have a larger specific surface area, higher surface energy and high surface lattice defects, the noble metal nano-materials have unparalleled excellent catalytic activity and selectivity. [2]

Here, we designed a Au@Pt nanoparticles with high catalytic activity and use it to detect ascorbic acid. In the human body, ascorbic acid is a highly effective antioxidant used to reduce the oxidative stress of ascorbate peroxidase. There are many important biosynthetic processes that also require the participation of ascorbic acid.

Ascorbic acid has a very important role. First of all, it can promote the absorption of iron and play a very good role in preventing iron deficiency anemia. In addition, ascorbic acid has an antioxidant effect, which can inhibit free radicals from oxidative damage to the human body and prevent tumors. Ascorbic acid can also participate in the synthesis of collagen to delay skin aging, make skin and blood vessels more elastic, and prevent heart diseases such as atherosclerosis. So ascorbic acid is a very important substance in the human body. [3]

2. Results and discussion

Preparation of Au@Pt nanocomposites

Stir 10 mL (1 mM) HAuCl₄ aqueous solution until boiling and add 1 mL (38.8 mM) sodium citrate into the vortex of the solution quickly to change the color from pale yellow to purplish red, continue boiling for 10 minutes, then stop heating, continue to stir down to room temperature. The resulting solution was centrifuged, washed by water for 3 times and dispersed in 8 mL deionized water. The mixture solution was stirred and heated to 80 °C. Then, H₂PtCl₆ aqueous solution (10 mM,1 mL) and

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 $NaBH_4$ aqueous solution (40 mM,1 mL) was added into the mixture quickly. Stir the solution at 80° C for 10 min, then stop heating and continue stirring to room temperature. The resulting solution was centrifuged, washed by water for 3 times and dispersed in 5 mL deionized water. [4] [5]

Characterization of Au@Pt nanocomposites

Transmission electron microscopy (TEM) image (Fig. 1) revealed that the average size of Au@Pt nanoparticles is 15-20 nm.

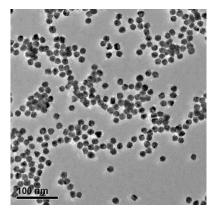


Figure 1: Representative TEM images

The influence of solution pH

Prepare 10 mM phosphate buffer solution with pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 40 mL per group and 20 mM 3,3',5,5'-tetramethylbenzidine (TMB) in DMSO solution. Then prepare 50 mM H_2O_2 aqueous solution. Take 0.6 mL of the pH 2-8 solution into a 1.5 mL centrifuge tube and 20 μ L of nanoenzyme sample were added. Then add 10 μ L of TMB and 10 μ L of H_2O_2 . The color change was observed. After 10 min, the absorbance of the solution at 652 nm was measured to observe the effect of pH on the enzyme activity and determine the optimal pH of the solution. Repeat 3 samples per group.

The influence of solution pH for the catalytic activity was studied. As shown in Fig. 2, Au@Pt nanocomposites had the highest absorbance in pH 4. That means when pH=4, Au@Pt nanocomposites had the best oxidase-like activity.

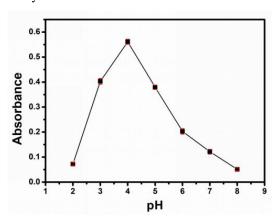


Figure 2: The different oxidase-like activities of different pH

The influence of temperature

Take 0.6 mL of the optimal pH solution and place it in a 1.5 mL centrifuge tube, then place them at 0, 10, 20, 30, 40, 50, 60, 70, and 80 degrees Celsius for 8 minutes, later add 20 μ L nanoenzyme sample, 10 μ L 50 mM TMB and 10 μ L 50 mM H₂O₂. Continue the constant temperature reaction and observe the color change. 10 minutes later, measure the absorbance of the solution at 652 nm to determine the optimal reaction temperature. Repeat 3 samples per group.

For comparison between different temperatures, we can obtain the information from Fig. 3 that the Au@Pt nanocomposites had the highest oxidase-like activity at 60°C.

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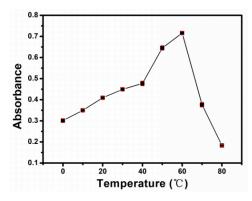


Figure 3: The different oxidase-like activities of different temperature

The influence of H₂O₂ concentration

Take 0.6 mL of the optimal pH solution and place it in a 1.5 mL centrifuge tube, keep at the optimal temperature for 8 min, add 20 μ L of nanoenzyme sample, and then add 0.5, 1, 2.5, 5, 10, 25, 50 mM H_2O_2 (final concentration), then add 10 μ L of TMB. Observe the color change and measure the absorbance of the solution at 652 nm after 10 minutes. Repeat 3 samples per group.

According to Fig. 4, we abserved the absorbance increases with the increasing of the concentration and remain unchanged at 10mM.

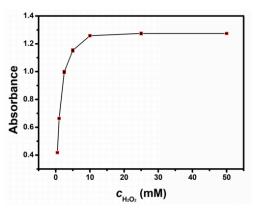


Figure 4: The different oxidase-like activities of different C_{H202}

The influence of TMB concentration

Take 0.6 mL of the optimal pH solution and place it in a 1.5 mL centrifuge tube, keep at the optimal temperature for 8 min , add 20 μ L of nanoenzyme sample, and then add 0.5, 1, 1.5, 2, 3, 4, 5 mM TMB (Final concentration), then add 10 μ L of H_2O_2 under optimal conditions. Observe the color change and measure the absorbance of the solution at 652 nm after 10 minutes. Repeat 3 samples per group. [6]

In this group of samples, Fig. 5 shows that the absorbance get the highest when the concentration of TMB is 2mM.

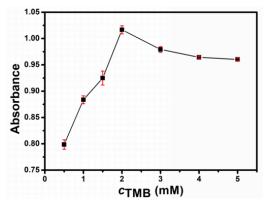


Figure 5: The different oxidase-like activities of different C_{TMB}

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Ascorbic acid testing

The experiment was performed under the optimal pH and temperature conditions. Take 0.6 mL of the optimal pH solution and place it in a 1.5 mL centrifuge tube, keep at the optimal temperature for 8 min, add 20 μ L of nanozyme sample and 10 μ L of H2O2 and TMB at the optimal concentration, and then add different concentrations of ascorbic acid. Continue the constant temperature reaction, observe the color change and measure the absorbance of the solution at 652 nm after 10 min. Repeat 3 samples per group. Determine the detection limit, detection range and linear range of ascorbic acid.

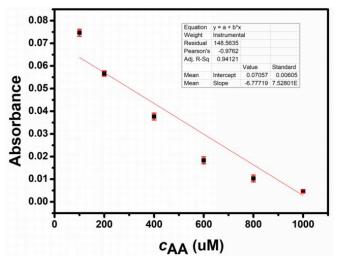


Figure 6

3. Conclusion

In summary, we have successfully prepared Au@Pt nanoparticles with excellent activity. As a mimic oxidase, Au@Pt nanoparticles show several advantages over natural enzymes and other existing alternatives. First, Au@Pt nanoparticles can be prepared easily in a short time and have low cost. Second, it is stable to heat, acid and alkali. Furthermore, it has higher catalytic efficiency. By taking advantage of Au@Pt nanoparticles, we can detect the content of ascorbic acid and perform corresponding treatments based on the content of ascorbic acid in the body. An increasing number of nanomaterials have been applied in catalyst. Therefore, to design and fabricate new multifunctional hybrid nanocomposites with high catalytic activity would be helpful to expand their applications in biomedical fields. [7]

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