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Synthesis of Cerium Oxide Nanorods and Detection of Hydrogen Peroxide

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Abstract

In recent years, nanomaterials have been widely used, and the concept of nano-enzyme has been gradually known by more and more people. However, the research on nano-enzyme is still at a relatively initial stage. Cerium oxide, as catalyst, can be used to catalyze various organic reactions, because of its good peroxidase activity, it has a good prospect in colorimetric detection. In this experiment, the rod-shaped cerium oxide nanomaterial was prepared by the REDOX reaction of cerium nitrate, and the influence of different conditions on the preparation of cerium oxide was studied. Finally, the decomposition performance of rod-shaped cerium oxide nano-enzyme for hydrogen peroxide was studied.

Keywords

Nanomaterials; Nano-enzyme; REDOX.

1. Introduction

In recent years, researchers studying in nanotechnology are fiercely seeking methods for exploring and applying nanoparticles and even nano-composites as sensors and detectors. For instance, in 2005, Soonwoo Chah' s team

prepared gold nanoparticles and tested that they can successfully detect protein as a simple colorimetric sensor. [1] As the field of nanotechnology is rapidly developing, researchers started to invest into the exploration of dumbbell-like nano-composites. For example. in 2013, Xiaolian Sun's team incorporated Pt48Pd52 and Fe3O4, justifying that the dumbbell-like nano-composites were more responsive to the reaction and produced much obvious color changes. [2] It is further proved that nanomaterials can be used for the detection of some trace substances.

Cerium dioxide (CeO2) nanoparticles, as the candidate of the substitution of HRP, has various applications. For example, they can be utilized in automotive industry as catalysts which help convert harmful gas carbon monoxide into carbon dioxide, which is less pernicious. What's more, cerium

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dioxide nanoparticles can also be used as polishing agents in semi-conductor industry. Thus, researchers proposed great interest and anticipation to cerium dioxide for replacing the expensive HRP. Pathikrit Saha's team conducted experiments with iron oxide and cerium oxide nanoparticles in December 2019, finding that cerium oxide incorporated with polyurethane scaffold was highly sensitive to hydrogen peroxide.[3] Furthermore, the solution can be recyclable for more than 10 assays without obvious loss, indicating cerium oxide is feasible for substituting HRP.[3] Cerium dioxide nanorods have large specific surface area and high electron transfer efficiency, which makes their ability in trace substances detection worth expecting.

Hydrogen Peroxide (H2O2) is known as the key of intracellular signaling transduction and cell growth. [2] It is reported that a large amount of hydrogen peroxide is produced by the immune system in response to bacteria invasion. [4] However, the excessive amount of hydrogen peroxide may lead to cell damages, which result in ageing, cancer, and other diseases. [4] The traditional detection method for hydrogen peroxide assay mainly utilizes a natural enzyme Horseradish Peroxidase (HRP) for hydrogen peroxide assay. [2] Despite the distinctive selectivity and sensitivity in catalzting the hydrogen oxide reduction, the tedious preparation and purification process and the tendency to denatured in assay conditions convince researchers to seek for substitutions for HRP on hydrogen peroxide assay. [2] In recent year, nanoparticles (NPs) draws increasing amount of attentions as novel enzyme mimics owing to the its relatively simple preparation and high stability. [5]

Therefore, Considering the catalytic effect of nanoenzyme, a method using cerium dioxide nanorods to detect hydrogen peroxide was first proposed. The main focus of this research is what assay condition the cerium dioxide nanorod can be the most responsive and produce the most significant outcome, so the pH of the solutions, temperature, and the concentrations of hydrogen peroxide and TMB are carefully investigated.

2. Experiment

2.1 Synthesis of CeO2:

First, 0.654 g Ce(NO3)3 6H2O was dissolved in 10mL ultra-pure water in 50mL round-bottom flask. Then 20mL sodium hydroxide(10 M) was dropped into the flask and stirred vigorously at room temperature. After that, the white slurry was transferred to the 50 mL stainless steel autoclave and stored at 100 ℃ for 24 hours. After the reaction, the solution was centrifuged and washed with deionized water until the solution reached neutral. Finally, dried the reactant at 120 ℃ in air for 12 hours to obtain the yellow CeO2 nanorods powder.

2.2 Characterization:

The morphologies of the product were studied using a transmission electron microscope (FEI Tecnai G2 20S-TWIN) The defective nanorods powder was eliminated and the solution was reconfigured until the nanorods reached a stable and uniform form.

Detection of the effect of solution PH:

Different kinds of phosphate was dissolved in ultra-pure water to get a serious of phosphate buffer with gradient pH (2.0, 3.0,4.0,5.0,6.0,7.0 and 8.0) and same concentration (10 mM) at first. We prepared 40mL buffer solution at each concentration for use. After that, 12.1 mg 3,3,5,5-tetramethylbenzidine (TMB) was dissolved in 1 mL dimethyl sulfoxide (DMSO) to obtain dimethyl sulfoxide solution of TMB with the concentration of 20mM.Then dilute hydrogen peroxide solution (10 M) to obtain aqueous solution of hydrogen peroxide (50 mM)

0.6 mL solution with pH 2-8 was placed into 1.5 mL centrifuge tubes, and 20 μL nanoenzyme samples with same concentration were added, followed by adding 10 μL TMB and 10 μL H2O2 with the same suitable concentration to observe the change of color. After 10 min, the absorbance of the solution at 625 nm was determined to observe the effect of solution pH on the enzyme activity and determine the optimal pH value for the solution reaction. Repeat 3 samples per group.

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2.3 Detection of the effect of solution temperature:

After that, 0.6 mL solution with pH 4 was placed into 1.5 mL centrifuge tubes and was incubated at different constant temperatures (0 ℃,10 ℃,20 ℃,30 ℃,40 ℃,50 ℃,60 ℃,70 ℃ and 80 ℃) for 8 min. After that, 20uL nanoenzyme samples with same concentration were added, followed by adding 10 μL TMB and 10 μL H2O2 with same suitable concentration. The solution reacted at constant temperature and observe the change of color. After 10 min, the absorbance of the solution at 625 nm was determined to observe the effect of temperature on the enzyme activity and determine the optimal temperature for the solution reaction. Repeat 3 samples per group.

2.4 Detection of the effect of hydrogen peroxide concentration:

Then, 0.6 mL solution with pH 4 was dropped into 1.5 mL centrifuge tubes and was incubated at 60 ℃ for 8 min. After that, 20 μL nanoenzyme samples with same concentration were added, followed by separately adding H2O2 with different concentration (0.5 mM, 1 mM, 2.5 mM, 5 mM, 10 mM, 25 mM, 50 mM). Then 10 μL TMB with suitable concentration was added and the change of color was observed. After 10 min, the absorbance of the solution at 625 nm was determined to observe the effect of hydrogen peroxide concentration on the enzyme activity. Repeat 3 samples per group. Plot a serious of points of H2O2 concentration and enzyme activity on the graph to explore the H2O2 concentration range that the enzyme activity of nanorods were positive linear correlation with hydrogen peroxide concentration.

2.5 Detection of the effect of TMB substrate concentration:

Finally, 0.6 mL solution with pH 4 was dropped into 1.5 mL centrifuge tubes and was incubated at 60 ℃ for 8 min. 20 μL nanoenzyme samples with same concentration were added after that followed by adding TMB with different concentration (0.5 mM, 1.0 mM,1.5 mM, 2.0 mM, 3.0 mM, 4.0 mM, 5.0 mM) separately. Then 10 μL H2O2 (10 mM)

with same concentration was added. The solution reacted at constant temperature and observe the change of color. After 10 min, the absorbance of the solution at 625 nm was determined to observe the effect of TMB substrate concentration on the enzyme activity and determine the optimal TMB substrate concentration for the solution reaction. Repeat 3 samples per group.

2.6 Detection of the suitable concentration of nanoenzyme concentration:

After that, 0.6 mL solution with pH 4 was placed into 1.5 mL centrifuge tubes and was incubated at 60 ℃ for 8 min. After that, 20uL nanoenzyme samples with gradient concentration (1 μg/ml, 2 μg/ml, 3 μg/ml, 4 μg/ml, 5 μg/ml, 6 μg/ml, 7 μg/ml) were added, followed by adding 10 μL TMB (2 mM) and 10 μL H2O2 (10 mM). The solution reacted at constant temperature and observe the change of color. After 10 min, the absorbance of the solution at 625 nm was determined to observe the effect of nanoenzyme concentration on the enzyme activity and determine the optimal nanoenzyme concentration for the solution reaction. Repeat 3 samples per group. Plot a serious of points of nanoenzyme concentration and enzyme activity on the graph to explore the nanoenzyme concentration range that the enzyme activity of nanorods were significantly changed with the increase of nanoenzyme concentration.

2.7 Test of unknown hydrogen peroxide sample:

To start with, repeat the experiment of H2O2 concentration detection under optional conditions and a chart related to absorbance and H2O2 concentration with the fitted equation (detection range H2O2 concentration ≤ 10 mM) was drawn for subsequent comparisons. Then, 0.6 mL solution with pH 4 was placed into 1.5 mL centrifuge tubes and was incubated at 60℃ for 8min.Then, 20 μL nanoenzyme with concentration 5 μg/ml were added, followed by adding 10 μL TMB (2 mM) and 10 μL solution sample. The solution reacted at constant temperature and observe the change of color. After 10 min, the absorbance of the solution at 625 nm was determined. Repeat 3 times and take the average. The average absorbance obtained was compared with the function in image to obtain the hydrogen

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peroxide concentration of the sample. If the absorbance is found to be beyond the detection range, the test was repeated after diluting the sample proportionally to obtain the accurate results.

3. Results and discussion

3.1 Characterization of CeO2 nanorods nanocomposites

it can be seen that the nanostructure of the sample is rod-like and the shape of the rod is relatively slender, as shown in the figure. The nanorods are relatively uniform with a length of several hundred nanometers. In TEM, the oxidation results show that the nanorods have good crystallinity and no obvious defects.

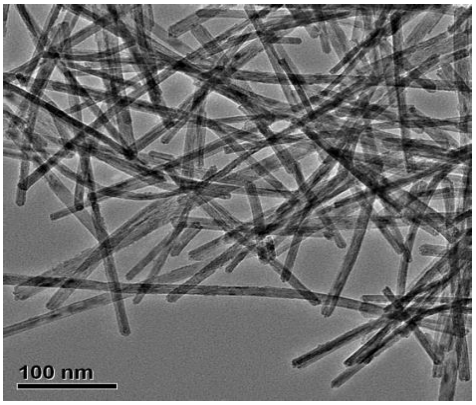

Fig. 1 Representative TEM images

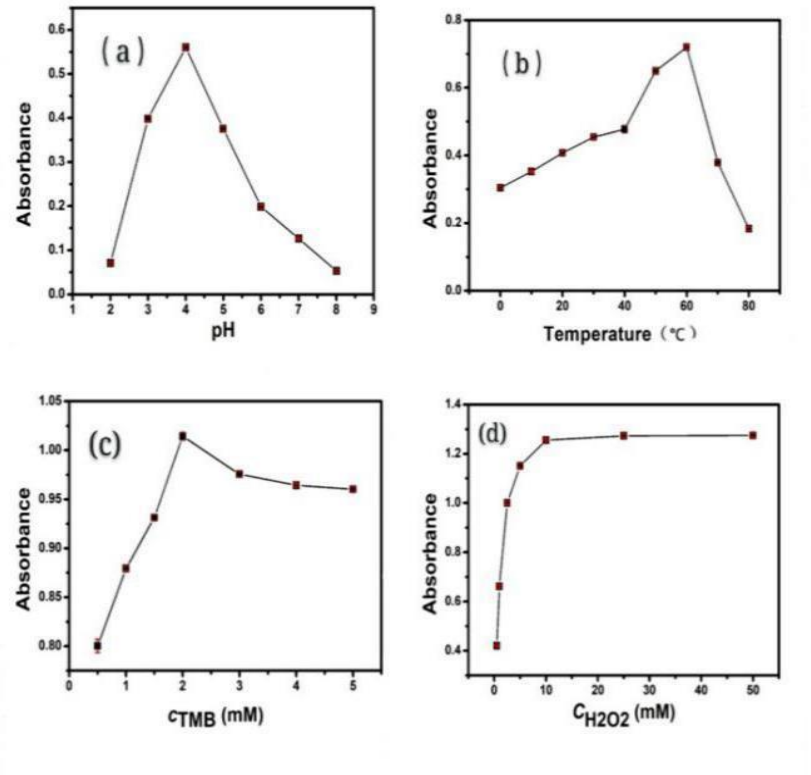

Fig. 2 Dependence of the peroxidase-like activity of CeO2 nanorods on (a) pH, (b) temperature, (c) TMB concentration and (d) H2O2 concentration

3.2 Enzyme-like activity of CeO2 nanocomposites

The catalytic activity of CeO2 nanorods also depends on pH, temperature, substrate and hydrogen peroxide concentration, just like other Nanoscale enzyme sample. CeO2 nanomaterials in the

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presence of phosphate buffer and hydrogen peroxide, substrate 3,3',5,5' - tetramethylbenzidine (TMB) DMSO solution will occur color reaction. The above results showed that CeO2 nanorods had the activity of intrinsic oxidase. The absorbance of the solution at 652 nm was determined, and the pH value had a great influence on the enzyme activity, and the optimal pH value was 4. As shown in Fig. 2 a. Nanoscale enzyme sample TMB and H2O2 were added to continue the constant temperature reaction to produce color changes. the absorbance of the solution at 652 nm was determined. As shown in Fig. 2 b. The catalytic activity is related to the concentration of substrate and hydrogen peroxide. At the optimum pH value of 4 and temperature of 60, the mixture was incubated and reacted with hydrogen peroxide at different concentrations in the presence of TMB. As shown in Fig.2 cd. In addition, the changes of nano-enzyme CeO2 concentrations were also studied. As shown in Fig.3, When the CeO2 concentration is 6(ug/mL), the absorbance reaches the maximum and remains unchanged afterwards.

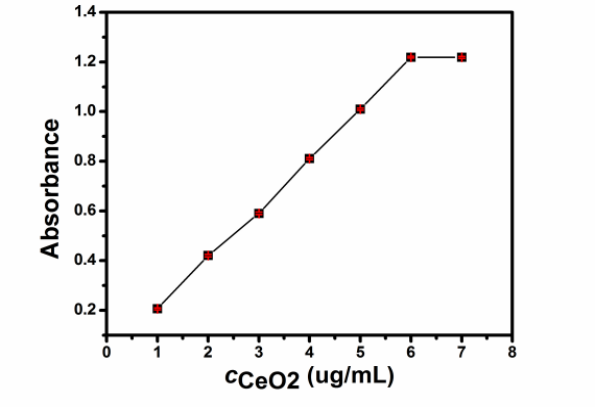

Fig. 3 Dependence of the peroxidase-like activity of CeO2 nanorods on CeO2 concentration

3.3 Production of CeO2 nanorods and its detection of Hydrogen peroxide

Tetravalent cerium solution is orange, the color is deep, tetravalent cerium oxide sex is extremely strong in acid solution, it can put the hydrogen peroxide into oxygen, its reduction for trivalent cerium, but ceria oxidizing under neutral and alkaline environment is weak, not easy to be restored, on the contrary, neutral or alkaline environment, trivalent cerium cerium oxide and cerium oxide (hydrogen) easy to oxidation, the hydrogen peroxide that it can be oxidized to ceria. Cerium oxide has good oxygen storage and release capacity, and Ceion is easy to be converted between +3 valence and +4 valence, and its contact area with the surface is relatively large, which makes it easier to react. The average absorbance obtained was compared with the function in image to obtain the hydrogen peroxide concentration of the sample. If the absorbance is found to be beyond the detection range, the test was repeated after diluting the sample proportionally to obtain the accurate results. Through the above reactions, hydrogen peroxide can be better detected, and the detection limit, detection range and linear detection range of hydrogen peroxide are determined. As shown in Fig. 4 (a,b). The oxidation of substrates is related to hydrogen peroxide, which can be better used in the detection of hydrogen peroxide. Ultra-pure water refers to a high degree of ion removal, usually refers to the electrical conductivity, and ultra-pure water has a low ion content. Due to low overpotential and rapid reaction kinetics, metals and their oxides are the most effective and stable electrolytic water catalysts. However, by catalyzing hydrogen peroxide generated by itself under acidic conditions through cerium ion, 3,3',5 '-tetramethylbenzidine can be oxidized by highly active hydroxyl radical (•OH) to produce colorless to blue color reaction. The maximum blue absorbance can be detected by scanning 652 nm with ultraviolet spectrophotometer. The absorbance can explain the effect of producing hydroxyl radical by nanocatalyst. The catalysis of hydroxyl radical is of great significance for the application of metal peroxides in biology, medicine and detection fields.

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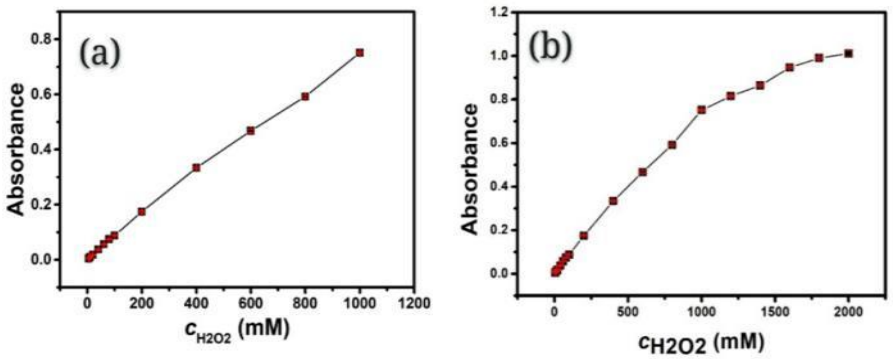

Fig. 4 (a) Hydrogen peroxide linear detection range, (b)Actual hydrogen peroxide detection

4. Conclusion

In summary, CeO2 nanorods have excellent catalytic oxidation activity, stability and oxidation-like activity. Under acidic conditions, CeO2 can promote the decomposition of H2O2 to produce hydroxyl radicals with stronger oxidizing ability, thereby oxidizing the color substrate and achieving the purpose of detecting hydrogen peroxide. At the same time, the unique composition of CeO2 nanoparticles (ce3+ and Ce4+ exist at the same time), when cerium switches between the two valence states, it has strong oxygen storage and release capabilities and charge exchange capabilities. Therefore, CeO2 nanorods can also be used in fields such as photocatalysis, supercapacitors, and lithium-ion batteries. The oxidation-like activity of CeO2 nanorods can also be used to detect cancer cells instead of H2O2, because most detectors need the catalytic effect of H2O2, which is harmful to human cells and their surrounding environment, and the use of CeO2 nanorod will reduce the damage of human cells. Otherwise, CeO2 nanorod can be used to solve the problems of the tumor catalytic therapy as CeO2 nanorod can overcome the lack of oxygen of the inner condition of human body. At present, the production process of CeO2 nanorods is becoming more and more mature, and industrial production is gradually realized. In view of the wide application fields of CeO2 nanorods, it has good prospects and economic value.

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