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Research Article

A Novel Method Using Flower-like Manganese Oxide Nanozymes for Colorimetric Detection of Ascorbic Acid

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Abstract: This paper proposes a method of utilizing a flower-like MnO_x nanozyme to conduct a colorimetric detection of ascorbic acid. The nanozyme is obtained by a chain of reaction of $K_3[Fe(CN)_6]$, $MnSO_4 \cdot H_2O$, polyvinyl pyrrolidone (PVP), NH_4F, ethanol, and water. During the experimental process, the flower-like nanozyme is added to the mixed solution, including phosphate buffer, H_2O_2, and 3,3',5,5'-tetramethylbe nzidine (TMB). The optimum reaction condition as following: pH 3.0, 30 μL 500mM H_2O_2, 25 μL 92 mM TMB, and 30 μL 0.1mM nanozyme. Under the optimum condition, the detection range is 2 - 26mM, and the linear detection range is 2 - 20mM.

Keywords: Colorimetric detection, Ascorbic acid, Flower-like Manganese Oxide Nanozymes

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1 Introduction

Natural enzymes with high efficiency have been widely investigated and applied in real practices. However, natural enzymes suffer from the high risk of instability and inactivation due to their high susceptibility to the exterior factors, including temperature, pH value, substrate concentration, etc. A novel natural enzyme mimetic called "nanozyme" has gradually became a supplant of natural enzyme with abundant advantages, owning to its high stability, low cost for preparation and storage. Characteristics including high specific surface area and customizable functions contribute to its reactivity, which, in turn, make nanozymes suitable to be used in biosensing, disease treatment, and immunoassay (D. L. Nelson, M. M. Cox, 2005). Among all the widely used nanozymes, Prussian blue nanoparticles (PBNPs) and its analogue are outstanding due to their ability to enhance the speed of electron transfer reaction to achieve electrochemical catalytic effect. Researchers around the world have developed a variety of usage of Prussian blue, including glucose detection and antioxidant. As (Zhang, X. Q., et al. 2010) reported, Prussian blue and its analogues possess the peroxidase-like activity. However, recent studies mainly placed their focus on the advantage of Prussian blue in colorimetric sensing in the existence of peroxide.

2 Experimental procedure

The detection of ascorbic acid includes following steps:

The optimum pH value, concentration of H_2O_2, flower-like nanozyme, and TMB were determined. 0.6 mL of phosphate buffer with optimal pH was placed in a 1.5 mL centrifuge tube, and different volume of H_2O_2, nanozyme, and TMB with optimal concentration were added subsequently. After 12 minutes, AA with different concentration was added, keeping reacting for 8 minutes. Then, the absorbance of the solution at 652 nm was measured. The whole process repeated for 2 times. The detection range of AA was determined

2.1 Synthesis of flower-like MnOx nanozyme

$MnSO_4 \cdot H_2O$ and PVP were dissolved in a mixture of 10 mL deionized water and 10 mL ethanol, denoted as solution 1. $K_3[Fe(CN)_6]$ was dissolved in 10 mL deionized water, denoted as solution 2. Under magnetic stirring, solution 2 was added dropwise into the solution 1, continuing to magnetic stir for 2 hours. The precipitation was obtained and dispersed in a mixture of 10 mL ethanol and 10 mL deionized water. NH_4F solution was added into the mixture as mentioned earlier while keeping stirring for 20 min. After being centrifuged, washed and dried, nanozyme could be obtained.

2.2 Preparation of flower-like MnOx nanocomposites

0.05g $MnSO_4 \cdot H_2O$ and 0.25g PVP was dissolved in a mixture of 10 mL ethanol and 10 mL deionized water, recorded as solution 1. 0.07g $K_3[Fe(CN)_6]$ was dissolved in 10 mL deionized water, recorded as solution 2. As the solution 1 was stirred, solution 2 was added dropwise to solution 1 under magnetic stirring for 2 hours. Subsequently, the mixture was washed with a solution mixed with ethanol and water (ratio of volume equals to 1:1) for 3 times by repeated centrifugation with 5000 rpm for 8 min. The precipitation was obtained and dispersed in a mixture of 10 mL of ethanol and 10 mL of deionized water. 1g NH_4F was dissolved in 8 mL of water. NH_4F solution was quickly added into the mixture as mentioned earlier while keeping stirring. Continuously, the mixture was stirred at room temperature for 20 min and respectively washed with water and ethanol by repeated centrifugation for 2 times. Finally, the mixture was vacuum-dried at 60 °C.

3 Experimental influencing factors

3.1 Effects of pH value

0.6 mL of phosphate buffer with different pH value (pH 3.0-10.0) was added into a 1.5mL centrifuge tube. Subsequently, 25 μL TMB, 20 μL Flower-like nanozyme sample, and 30 μL H_2O_2 were added. The solution was kept reacting at the room temperature, and the absorbance could be determined at 652 nm after 8 minutes. Overall, the whole process repeated for 2 times. As shown in Figure 3, the optimal concentration of 92.0 mM TMB was determined for Flower-like nanozyme in the following experiments.

3.2 Effects of H_2O_2 concentration

0.6 mL of phosphate buffer (pH 3.0) was put into a 1.5mL centrifuge tube. After that, 30 μL H_2O_2 with different concentration of 83.33, 166.67, 250.00, 333.33, 416.67, 500.00 mM, 30 μL Flower-like nanozyme sample, and 25 μL 92.0 mM TMB were added. The solution was kept reacting at the room temperature, and absorbance could be determined at 652 nm after 8 minutes. The whole process repeated for 2 times. As shown in Figure 4, the optimal concentration of 500.00 mM H_2O_2 was chosen for flower-like nanozyme in the following experiments.

3.3 Effects of TMB concentration

0.6 mL of phosphate buffer (pH 3.0) was put into a 1.5mL centrifuge tube. 25 μL TMB with different concentration of 5.0, 10.0, 20.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 mM, 20 μL Flower-like nanozyme sample, and 30 μL H_2O_2 were added. The solution was kept reacting at the room temperature, and absorbance could be determined at 652 nm after 8 minutes. The whole process repeated for 2 times. As shown in Figure 5, the optimal concentration of 92.0 mM TMB was determined for Flower-like nanozyme in the following experiments.

3.4 Effects of flower-like MnO_x nanozyme concentration

0.6 mL of phosphate buffer (pH 3.0) was put into a 1.5mL centrifuge tube. After that, 10 μL Flower-like nanozyme sample with different concentration of 0.033, 0.05, 0.066, 0.083, 0.100mM, 30 μL 500.00 mM H_2O_2 and 25 μL 92.0 mM TMB were added. The solution was kept reacting at the room temperature, and absorbance could be determined at 652 nm after 8 minutes. The whole process repeated for 2 times. As shown in Figure 5, the optimal concentration of 0.1 mM Flower-like Nanozyme was chosen in the following experiments.

4 Detection of ascorbic acid

0.6 mL of phosphate buffer (pH 3.0) was put into a 1.5mL centrifuge tube. 30 μL Flower-like nanozyme sample, 30 μL 500 mM H_2O_2, and 25 μL 92.0 mM TMB were added. AA with different concentration 2.0, 4.0, 8.0, 12.0, 16.0, 18.0, 20.0, 22.0, 24.0, 26.0 mM were respectively added after 12 minutes. The color changes of solution were observed after 9 minutes, and the absorbance could be determined at 652 nm (Figure 7). As Figure 8 illustrates, absorbance is linearly correlated to AA concentration from 2 mM to 20 mM. In order to evaluate the applicability and accuracy of the proposed

method, the real sample of AA concentration was measured. The theorical value of concentration of the sample is 5.45 mM, and the value in our measurement is 5.38 mM. Our result has a 0.013% (< 0.05%) margin of error. AA concentration of the real sample based on the detection curve was proved to be consistent with the concentration shown in the ingredients.

5 Conclusion

This detection method aims to find a viable, simple way for colorimetric detection of detecting ascorbic acid (AA) by using flower-like manganese oxide nanozyme. Nanozyme is synthesized by using the coprecipitation method. The optimal catalytic parameters, including pH, substrate concentration, 3,3',5,5'-tetramethylbenzidine (TMB) concentration, and H_2O_2 concentration, etc., were obtained based on testifying the absorbance which can monitor the catalytic reactivity vividly. It is proved that this novel nanozyme possesses the ability to detect AA with high efficiency and accuracy.

References

[1] D. L. Nelson and M. M. Cox, Lehninger Principles of Biochemistry, vol. 6, chapter 6, W. H. Freeman, New York, NY, USA, 2005.

[2] Zhang, X. Q.; Gong, S. W.; Zhang, Y.; Yang, T.; Wang, C. Y.; Gu, N. Prussian blue modified iron oxide magnetic nanoparticles and their high peroxidase-like activity. J. Mater. Chem. 2010, 20:5110–6.