Research Article

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Inhibitory and Stability Studies on Graphene Oxide Gold Nanozymes

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Abstract

In this study, peroxidase-like graphene oxide/gold nanoparticles were synthesized and characterized for their biochemical stability including pH and thermal stability by determining their relative activity as an index for their stability monitoring. Besides, the inhibitory effect of metal ions on their enzyme like activity was also studied. The results of thermal stability studies revealed a maximal activity over a wide temperature range of 20-40 ℃, revealing high thermal stability of the as-synthesized peroxidase-like graphene oxide/gold nanoparticles. The pH stability measurements revealed a maximal activity over pH= 4.5-5.5 for the as-synthesized peroxidase-like graphene oxide/gold nanoparticles, showing their wide pH range. Moreover, the inhibitory effect of silver ions (as a model metal ion) on their activity was checked at optimal pH and temperature, revealing that the activity of nanozymes was decreased by increasing the concentration of silver ions and reached about 63% upon the addition small portion of silver ions into the reaction media.

Keywords: Inhibitory Effect, Ph Stability, Thermal Stability, Graphene Oxide Gold Nanoparticles

1. Introduction

Despite the high specificity, selectivity, and catalytic performances of the native enzymes, they suffer several disadvantages such as narrow pH and thermal range which limits their practical applications [1-10]. The traditional way for solving these problems is the immobilization of enzymes to enhance their stability and recoverability [10-15]. However, with the fast development of nanoscience in recent years, a wide veracity of nanomaterials with unique spectral, optical, catalytic, and stability has been introduced [16-23]. Among these nanostructures reveal significant enzyme-like activity for instance peroxidase-like, oxidase-like, urease-like, and catalase-like, etc. [24-35]. These enzyme-like nanoparticles show significant advantages over native enzymes for instance, high pH and thermal stability, excellent reusability, and high storage stability, making them the best alternative of enzymes for proceeding with enzyme-mediated process [36-44]. Nanozymes have been used for different applications such as dye degradation and the battery industry, as well as, sensing and detection [45-57], especially after the first report of COVID-19 [58, 59], they applied for its clinical sensing [60-67]. Herein, peroxidase-like graphene oxide/gold nanoparticles were synthesized and characterized for their peroxidase-like activity. Considering the significant effect of pH and temperature on the activity of enzyme/nanozymes, the pH and thermal stability of the peroxidase-like graphene oxide/gold nanoparticles were evaluated by determining their relative activity as an index for their stability monitoring. Besides, the inhibitory effect of silver ions on their activity was checked at optimal pH and temperature.

2. Experimental

2.1. Synthesis of Peroxidase-Like Graphene Oxide/Gold Nanoparticles

For the preparation of graphene oxide gold nanoparticles, the gold nanoparticle and graphene oxide dispersions were mixed with a 1:1 ratio. The reaction mixture was then ultra-sonicated for about 30 minutes to produce the graphene oxide gold nanoparticles. The obtained graphene oxide/gold nanoparticles dispersion was then stored in the dark for further use.

2.2. Enzyme Assay

TMB assay was used for determining the enzyme-like activity of peroxidase-like graphene oxide/gold nanoparticles as a standard method. Briefly, 200 µL of 0.2 mg/mL nanozyme was added to 6.8 mL of 0.2 M acetate buffer (pH 4.5). Then, 1.0 mL TMB (5 mM) and 1.0 mL H2O2 (0.2 M) were added to the solution. After reacting for 10 minutes, the absorbance of the colored product was measured at 650 nm.

3. Results and Discussion

Considering the significant effect of pH and temperature on the activity of enzyme/nanozymes, the pH and thermal stability of the peroxidase-like graphene oxide/gold nanoparticles were evaluated by determining their relative activity as an index for their stability monitoring.

3.1. Effect of pH

The effect of pH on the enzyme-like activity of the as-prepared peroxidase-like graphene oxide/gold nanoparticles was investigated by probing their relative activity by changing the pH of the reaction media. The relative activity of nanozymes was determined using the following equation;

Relative activity = (Activity/Maximal Activity) × 100

The results are shown in Figure 1. This figure shows the relative activity of enzymes as a function of the pH of the media. As can be seen from this figure, the relative activity of nanozymes increased by increasing pH from 3.0, reached the maximal value at 5.0, and then decreased by increasing the pH. It is notable that, the pH stability measurements revealed a maximal activity over pH= 4.5-5.5 for the as-synthesized peroxidase-like graphene oxide gold nanoparticles.

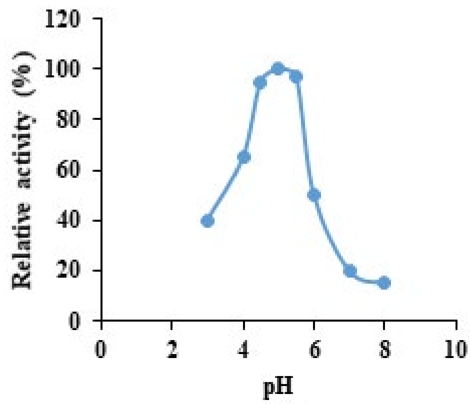

Figure 1: Effect of pH on the enzyme-like activity of the as-prepared peroxidase-like graphene oxide gold nanoparticles

3.2. Effect of Temperature

The effect of temperature on the enzyme-like activity of the as-prepared peroxidase-like graphene oxide/gold nanoparticles was investigated by probing their relative activity by changing the operating temperature. The relative activity of nanozymes in different temperatures was determined using the following equation;

Relative activity = (Activity/Maximal Activity) × 100

The results of this investigation are shown in Figure 2. This figure shows the relative activity of the enzyme as a function of the temperature of the reaction media. As can be seen from this figure, the relative activity of nanozymes increased by increasing temperature from 4.0 ℃, reached maximal value, and then decreased by increasing the operating temperature. It is notable that, the results of thermal stability studies revealed a maximal activity over a wide temperature range of 20-40 ℃, revealing high thermal stability of the as-synthesized peroxidase-like graphene oxide/gold nanoparticles.

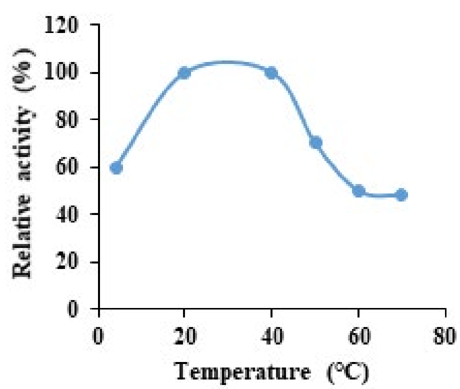

Figure 2: Effect of temperature on the enzyme-like activity of the as-prepared peroxidase-like graphene oxide gold nanoparticles

3.3. Inhibitory Effect of Silver Ions

The inhibitory effect of silver ions on their activity was checked at optimal pH and temperature. To do this, different amounts of silver ions were added into the solution nanozymes, followed by incubation at room temperature for 30.0 min to complete the inhibition process. Thereafter, the enzyme assay was carried out to determine the nanozyme activity. The results were used for constructing the histogram of nanozyme activity as a function of inhibitor concentration (Figure 3). The results of Figure 3 revealed that the activity of nanozymes was decreased by increasing the concentration of silver ions and reached about 63% upon the addition small portion of silver ions into the reaction media. Hence, silver ions can significantly inhibit the enzyme-like activity of the as-prepared peroxidase-like graphene oxide/gold nanoparticles.

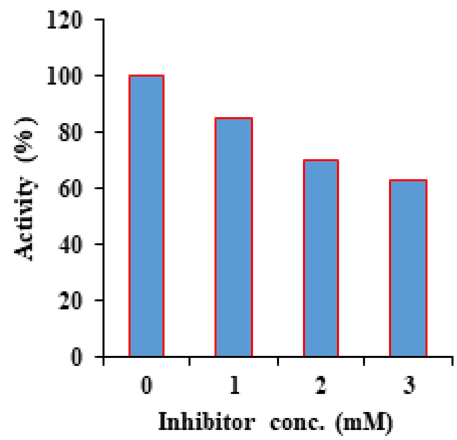

Figure 3: Inhibitory effect of silver ions on the enzyme-like activity of the as-prepared peroxidase-like graphene oxide gold nanoparticles.

4. Conclusions

Herein, peroxidase-like graphene oxide/gold nanoparticles were synthesized and characterized for their peroxidase-like activity. Considering the significant effect of pH and temperature on the activity of enzyme/nanozymes, the pH and thermal stability of the peroxidase-like graphene oxide/gold nanoparticles were evaluated by determining their relative activity as an index for their stability monitoring. The results of thermal stability studies revealed a maximal activity over a wide temperature range of 20-40 °C, revealing high thermal stability of the as-synthesized peroxidase-like graphene oxide/gold nanoparticles. Besides, the pH stability measurements revealed a maximal activity over pH= 4.5-5.5 for the as-synthesized peroxidase-like graphene oxide/gold nanoparticles. Moreover, the inhibitory effect of silver ions on their activity was checked at optimal pH and temperature, revealing that the activity of nanozymes was decreased by increasing the concentration of silver ions and reached about 63% upon the addition small portion of silver ions into the reaction media.

Acknowledgment

None.

Conflict of interest

None.

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