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Research Article

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Captopril Molecules Reveal Strong pH-, Temperature-, And Concentration-Dependent Inhibitory Effect on Nanozymatic Activity of Peroxidase-Like Nitrogen-Doped Carbon Dots

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Abstract

Herein, peroxidase-like nitrogen-doped carbon dots were synthesized via a simple hydrothermal method and then characterized for their FL properties and enzyme-like activity. Thereafter, the effect of captopril molecules on their nanozymatic activity was evaluated by calculating their nanozymatic activity in the presence and absence of captopril, revealing a strong inhibitory effect of captopril on the nanozymatic behavior of peroxidase-like nitrogen-doped carbon dots. The effect of pH on the inhibitory effect of captopril (2 mg L^{-1}) was evaluated over pH=2.0-6.0, revealing maximal and minimal inhibitory effect at pH=3.5 and pH=6.0, in order. The temperature-dependent inhibitory experiments exhibited a maximal inhibition percentage over 35-40 °C and a minimum inhibition percentage at 25 °C. Finally, the concentration-dependent inhibition was also checked in the presence of 0.0-8.0 mg L-1 of captopril, the results reveal that the relative activity of nanozymes was inhibited by increasing the inhibitor concentration and finally reached about 23% of its initial activity (i.e., 77% inhibition, inhibitor conc. of 8.0 mg L^{-1}).

Keywords: Peroxidase-Like Nitrogen-Doped Carbon Dots, Enzyme/Nanozymes Inhibitors, Captopril, pH-Dependent Inhibition, Concentration-Dependent Inhibition, Temperature-Dependent Inhibition.

1. Introduction

Nanozymes are a huge group of nanomaterials such as carbon nanomaterials, metal-based nanoparticles, metal oxides, metal-organic frameworks, and nanoclusters that exhibit intrinsic enzyme-like activity [1-12]. Among different nanozymes, most of them reveal significant peroxidase-like activity and cleavage of the peroxide bonds to produce active oxygen species such as hydroxyl radicals [13-18]. The produced radicals can then react with chromogenic substrates and oxidize them to their corresponding colored products. The spectrophotometric assay and recording of the absorbance of these products can be used as an index for calculating the nanozymatic activity of the nanoscale peroxidase-like materials [19-25]. Moreover, it is proved by several researches that the enzyme-like activity of the nanozymes can be inhibited by some inhibitors as same as the native enzymes. It is inhibitory effect can be used for several aims especially for sensing and detection toward developing both clinical and analytical protocols [26-30]. It is well known that among different identified enzymes, peroxidase enzymes, especially horseradish peroxidase (HRP), are attractive enzymes from both industrial and clinical points of view [28]. Regarding the peroxidase enzymes, hydrogen peroxide is the initiator of the peroxidase-mediated reactions and the oxidation of a wide range of organic compounds (substrates) including aromatic amines, phenols, and their mixtures can be initiated in the presence of hydrogen peroxide and peroxidase enzyme [28].

However, the peroxidase as same as other natural enzymes shows some of the following serious disadvantages such as pH and temperature instability, difficult recovery protocol, short storage time, no reusability, and highly expensive production methods. Hence, to fix these drawbacks, the immobilization of enzymes was proposed [31-33]. However, during most immobilization protocols, the enzyme's initial activity is reduced and some of them are expensive. Hence, a better solution is needed to overcome these difficulties, the new field of nanozymes is the right solution [28]. In fact, the fast development of nanoscience and material chemistry has -increased interest in researching new and innovative synthesis methods to produce new nanomaterials with unique catalytic activity unique optical properties high active area antibacterial properties and high biocompatibility [34-41]. Among different nanomaterials, nanozymes as nanomaterials with high enzyme-like activity can be used to simulate enzymatic reactions in harsh environmental conditions (for example, higher temperature or wider pH range) [1-28]. Hence, due to their high stability and intrinsic enzyme-like properties, the nanozymes were used for different applications, especially for constructing sensing assays for a wide variety of analytes, e.g., amino acids, glutathione (GSH), tetracycline, metal cations, glucose, H2O2, explosives, malathion and new SARS-CoV-2 as after the first report of COVID-19 [42-54]. However, the researches focusing on the inhibitory effect of inhibitors on nanozymes activity are limited to a few reports. Hence, in this continuation, the inhibitory effect of captopril molecules on the nanozymatic activity of peroxidase-like nitrogen-doped carbon dots were studied. In this regard, the carbon dots were synthesized via a simple solvent-free method and then characterized for their FL properties and enzyme-like activity. Thereafter, the effect of captopril molecules on their nanozymatic activity was evaluated by calculating their nanozymatic activity in the presence and absence of captopril. To explore more precise on the inhibitory effect of captopril, the effect of pH and temperature was also evaluated. Finally, the concentration-dependent inhibition was also checked to estimate the maximum inhibition percentage of nanozymes by introducing captopril into the reaction solution.

2. Experimental

2.1 Synthesis of Nanozymes

The peroxidase-like nitrogen-doped carbon dots were synthesized using ethylenediaminetetraacetic acid as both carbon and nitrogen sources. In a typical experiment, 300 mg ethylenediaminetetraacetic acid was directly heated at 400 ℃ for about 2 hours. Afterward, the CDs were dissolved in acetone and centrifuged to remove the residual solid particles. The solvent was then evaporated and the results CDs were collected and dissolved in water for next use.

2.2 Inhibitory Experiments

In a typical test, different concentrations of inhibitor were introduced into acetate buffer (pH, 4.0; 0.1 M) containing 60 µL nanozymes, 0.4 mM TMB, and 0.02 M hydrogen peroxide. The mixture was incubated for about 12 min to complete the oxidation process. Afterward, the absorbance of the oxidation product was calculated at 662 nm. Considering the ε (TMB-ox) = 39000 cm-1 M^{-1}, the reaction rate of the nanozymatic process was estimated. Besides, the residual activity of the nanozymes in the presence and the absence of the inhibitor molecules was calculated by dividing the activity of the nanozyme by the activity of control (i.e., activity in the absence of inhibitor) (Eq. 1).

$$Residual\ activity\ (\%) = \frac{\text{Activity in the presence of inhibitor}}{\text{Activity in the absence of inhibitor}} \times 100 \qquad Eq.(1)$$

3. Results and Discussion

3.1 Characterization of Nanozymes

The as-prepared enzyme-like nitrogen-doped carbon dots were characterized by investigating their FL behavior. In this regard, the FL spectrum of the as-prepared CDs was recorded upon an excitation wavelength of 350 nm. The results are shown in Figure 1. As can be seen from this figure, the as-prepared CDs have an FL spectrum over 360-550 nm with a λ_{max} at 404 nm, revealing successful synthesis of the enzyme-like nitrogen-doped carbon dots.

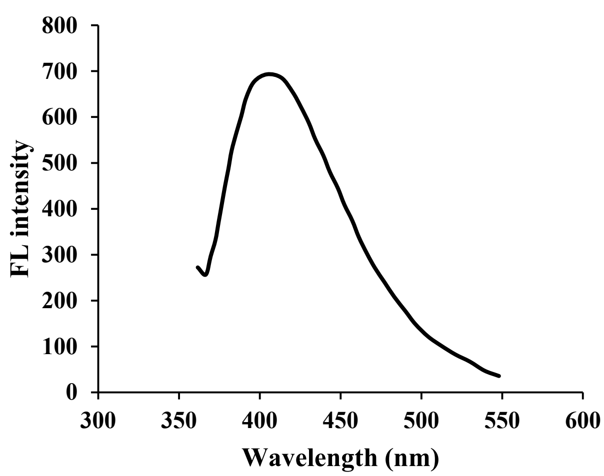


Figure 1: FL Spectrum of as-Prepared Nitrogen-Doped Carbon Dots.

3.2 Effect of pH on the Inhibitory Effect of Captopril on the Activity of CDs

The pH of the solution is one of the most important factors affecting the enzyme/nanozymes activity. Hence, the effect of the pH on the inhibitory effect of captopril on the nanozymatic activity of peroxidase-like nitrogen-doped carbon dots was investigated by probing their activity in the presence of a constant concentration of captopril (2 mg L^{-1}) as an inhibitor in a pH range over 2-6. Thereafter, the inhibitory effect of the inhibitor was calculated using the following formula;

$$Inhibition\ of\ activity\ (\%) = \frac{(A_0 - A)}{A_0} \times 100 \qquad Eq.(2)$$

Where A0 and A are represented by the absorbance at 652 nm in the absence and the presence of inhibitor, respectively. The plot of inhibition percentage as a function of pH is shown in Figure 2. The results of this figure revealed a pH-dependent inhibition of the enzyme-like activity of CDs by introducing captopril into the nanozymes solution. Considering Figure 2, the inhibitory effect of captopril was increased by increasing pH and reached its maximal value (about 20%, captopril (2 mg L^{-1})) at pH=3.5 and then decreased by increasing the pH of reaction media. It should be mentioned that the minimum inhibitory effect of captopril was observed at pH= 6.0 (only 3%).

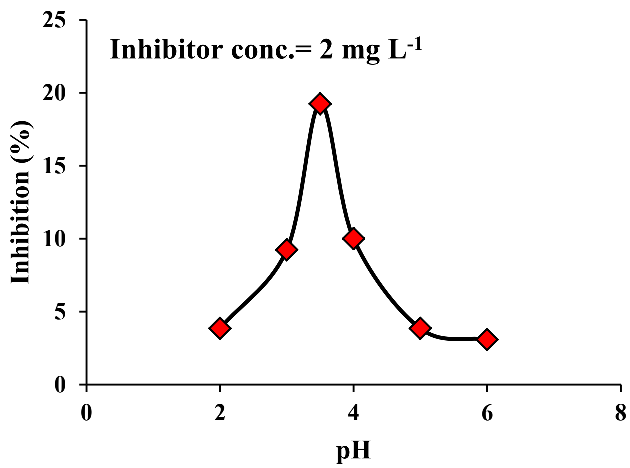


Figure 2: The Effect of pH on the Inhibitory Effect of Captopril on the Nanozymatic Activity of Peroxidase-Like Nitrogen-Doped Carbon Dots.

3.3 Effect of Temperature on the Inhibitory Effect of Captopril on the Activity of Cds

One of the most important factors affecting the enzyme/ nanozymes activity is the reaction temperature. Hence, the effect of the temperature on the inhibitory effect of captopril on the nanozymatic activity of peroxidase-like nitrogen-doped carbon dots was evaluated via probing their activity in the presence of a constant concentration of captopril (2 mg L^{-1}) in a temperature range of 25-45℃. The plot of inhibition percentage as a function of reaction temperature is shown in Figure 3. The results of this figure exhibited a temperature-dependent inhibition of the enzyme-like activity of CDs by introducing captopril into the solution. The inhibitory effect of captopril was increased by increasing temperature and reached its maximal value over 35-40 ℃ and then slightly decreased. Notably, the minimum inhibitory effect of captopril was observed at t= 25 ℃ (about 7%).

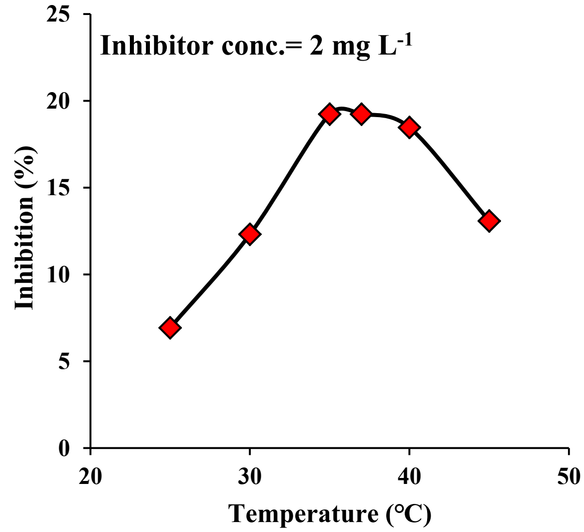
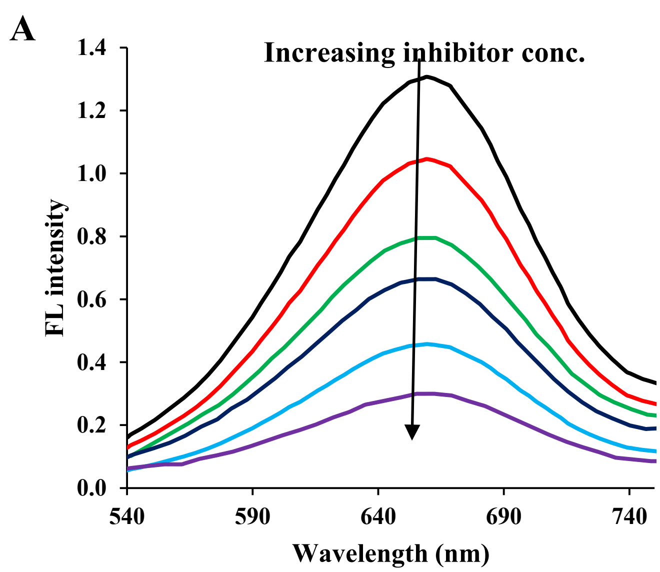


Figure 3: The effect of pH on the Inhibitory Effect of Captopril on the Nanozymatic Activity of Peroxidase-Like Nitrogen-Doped Carbon Dots.

3.4 Concentration-Dependent Inhibition

The concentration-dependent inhibition of the nanozymatic activity of peroxidase-like nitrogen-doped carbon dots was evaluated by calculating their nanozymatic activity in the presence and absence of different concentrations of captopril. The UV-visible spectra of the oxidation product of TMB in the presence and the absence of different concentrations of captopril as an inhibitor are shown in Figure 4A, revealing that the absorbance at 662 nm was significantly reduced by increasing the inhibitor concentration, showing the concentration-dependent inhibitory effect of captopril molecules on the CDs-mediated oxidation of TMB. However, to provide a better view of the inhibitory effect of captopril on the nanozymes activity, the residual activity of nanozymes was calculated as a reliable index (Figure 4B). The results reveal that the relative activity of nanozymes was inhibited by increasing the inhibitor concentration and finally reached 23% of its initial activity (i.e., inhibition percentage of 77%), revealing a strong inhibitory effect of captopril on the nanozymatic behavior of peroxidase-like nitrogen-doped carbon dots.



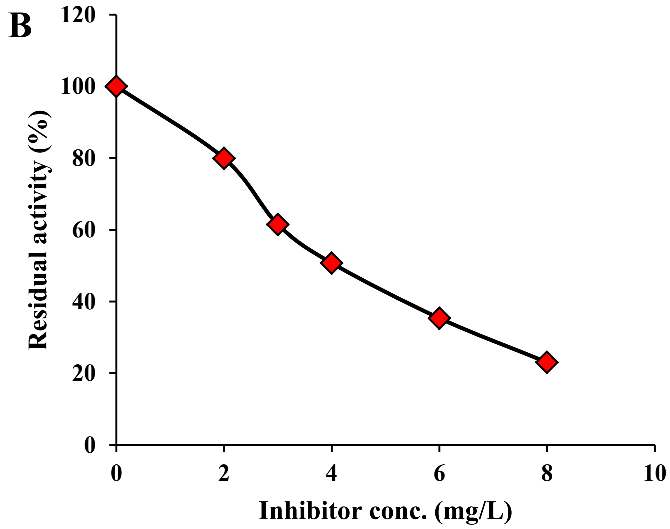


Figure 4: (A) UV-Visible Spectra of the Oxidation Product of TMB in the Presence and the Absence of Different Concentrations of Captopril as an Inhibitor, (B) Residual Activity of as-prepared Nanozymes as a function of Inhibitor Concentration.

4. Conclusions

Herein, peroxidase-like nitrogen-doped carbon dots were synthesized via a simple hydrothermal method and then characterized for their FL properties and enzyme-like activity. Thereafter, the effect of captopril molecules on their nanozymatic activity was evaluated by calculating their nanozymatic activity in the presence and absence of captopril, revealing a strong inhibitory effect of captopril on the nanozymatic behavior of peroxidase-like nitrogen-doped carbon dots. The effect of pH on the inhibitory effect of captopril (2 mg L^{-1}) was evaluated over pH=2.0-6.0, revealing maximal and minimal inhibitory effect at pH=3.5 and pH=6.0, in order. The temperature-dependent inhibitory experiments exhibited a maximal inhibition percentage over 35-40 ℃ and a minimum inhibition percentage at 25 ℃. Finally, the concentration-dependent inhibition was also checked in the presence of 0.0-8.0 mg L^{-1} of captopril, the results reveal that the relative activity of nanozymes was inhibited by increasing the inhibitor concentration and finally reached about 23% of its initial activity (i.e., 77% inhibition, inhibitor conc. of 8.0 mg L^{-1}).

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Conflict of Interest

There is no conflict of interest.

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