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Article

Investigating the Biochemical Stability and Kinetic Performances of SiO_2@AuNPs Nanocomposite as High Throughput Peroxidase Alternatives

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Abstract: In this study, considering the peroxidase-like activity of the SiO_2@AuNPs nanocomposite, their biochemical properties including, pH stability, cycling stability, shelf stability, and kinetic parameters were investigated. The results revealed that the as-mentioned SiO_2@AuNPs nanocomposite reveal its maximal activity at pH=4.0 along with saving 83.3% of its maximal activity at pH=5.0. Besides, the reusability and shelf-storage studies exhibited that the SiO_2@AuNPs nanocomposite retained 90% and 100% of its initial activity after 5 operational cycles and 30 days of storage, in order. The kinetic parameters of the as-prepared nanozymes were calculated using Menten kinetic model, revealing a V_{max} of 1.35 μM min^{-1} and a K_m as low as 0.06 mM for the SiO_2@AuNPs nanocomposite. Based on the results of this work, the as-prepared SiO_2@AuNPs nanocomposite with intrinsic peroxidase-like activity shows high substrate affinity and catalytic efficiency along with excellent cycling- and shelf-stability, making them suitable for application in peroxidase-mediated reactions instead of the native enzyme.

Keywords: SiO_2@AuNPs nanocomposite; intrinsic peroxidase-like activity; cycling stability; shelf stability

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1. Introduction

Importance and practical application of nanotechnology in modern life lead to design and synthesis of different nanomaterials with optical [1–3], catalytic [4,5], medical [6], anti-cancer features [7], or anti-bacterial [8,9] characteristics such as carbon and quantum dots [10,11], metal-based nanoparticles [12,13], magnetic nanoparticles [14], metal oxide nanoparticle [15], and metal-organic frameworks [16,17], etc. Among various nanoparticles with different properties, recently, nanomaterials with enzyme-like properties, called nanozymes have been widely utilized for catalyzing industrial, clinical, and environmental enzyme-mediated reactions under harsh conditions [18–21]. The most significant advantage of these nanozymes compared to the native enzymes is their lower cost, higher efficiency, and especially, their high cycling stability and recyclability [19,22]. Up to now, different nanoparticles with intrinsic peroxidase-like activity were designed and synthesized, for instance, Mn_3O_4 nanozymes [23], Cu-$CuFe_2O_4$ nanozymes [24], BSA-stabilized manganese phosphate nanoflower [25], BSA-stabilized manganese dioxide nanoparticles [26], silica-coated-magnetic nanoparticles [27], carbon nanozymes [28], MnO_2 nanoparticles [29], self-cascade pyrite nanozymes [30], Fe_3O_4 nanozymes [31], metal-organic frameworks [32], gold nanozymes [33], S/N codoped carbon nanozymes [34], and silver nanoparticles [35]. Among the different nanomaterials with excellent peroxidase-like activity, gold-based nanozymes had been widely for developing nanozyme-based sensors [36,37], nanozyme-based cancer treatment [38], and nanozyme-mediated dye degradation [39]. Hence, evaluation of their biochemical features and enzyme-like properties is important for developing nanozyme-based systems with better figures of merit. In this regard, recently, our research group reported a research article on the investigation of biochemical behaviors of BSA-stabilized gold nanoparticles [40]. Considering our best knowledge, there is no report on the specific enzyme-like activity, biochemical stability, and kinetic performances of SiO_2@AuNPs

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nanocomposites. Hence, herein, the biochemical properties (pH-, cycling-, & shelf- stability) and kinetics parameters of SiO_2@AuNPs nanocomposite as the high throughput peroxidase alternatives were evaluated. The as-synthesized nanocomposite was characterized by the TEM imaging method. Besides, the peroxidase-like activity of the as-prepared SiO_2@AuNPs nanocomposite was quantified using the standard peroxidase assay. Considering the high peroxidase-like activity of the SiO_2@AuNPs nanocomposite, their biochemical properties including, pH stability, cycling stability, shelf stability, and kinetic parameters were investigated. Based on the results of this work, the as-prepared SiO_2@AuNPs nanocomposite with intrinsic peroxidase-like activity shows high substrate affinity and catalytic efficiency along with excellent cycling- and shelf-stability, making them suitable for application in peroxidase-mediated reactions instead of the native enzyme.

2. Experimental

2.1. Synthesis of SiO_2@Au nanocomposite

In a typical experiment, 1.0 mL of $HAuCl_4$, 10.0 μL tetrakis(hydroxymethyl)phosphonium chloride, and 500.0 μL NaOH (2.0 M) were introduced in 50.0 mL deionized water. The resulting mixture was stirred for about 1.0 hour to prepare the colloidal gold solution. To synthesize the amino-functional SiO_2 NPs, 1.5 mL of TEOS was added into 40.0 mL of pure ethanol, followed by the addition of 3.0 mL of NH_4OH. The mixture was stirred for about 20.0 hours. Afterward, 20.0 mg of the resulting precipitate was treated with 600.0 μL of APTES for preparation of the final product. Afterward, 10.0 mg of the amino-functional SiO_2 NPs were incubated with a solution of colloidal gold (prepared in section 2.3) for about 12.0 hours. Afterward, the product was separated from the reaction media upon centrifuge at 10000 rpm for 30.0 min. The resulting product was dispersed in 1 mg mL^{-1} of PVP (stabilizer) aqueous solution, followed by the addition of 60.0 μL of $HAuCl_4$ (10.0 mM) and 120.0 μL of 10.0 mM ascorbic acid (reducing agent) into the reaction media. The synthesis process was followed by stirring the above-mentioned mixture for about 5 min. Afterward, the resulting SiO_2@AuNPs nanocomposite was collected, washed, and then dried at room temperature.

2.5. Nanozyme activity assay

In a typical assay, 100.0 μL of 1.0 mM TMB was introduced into 1.0 mL acetate buffer (pH=4.0) containing the SiO_2@Au nanocomposite, followed by adding 100.0 μL of 100 μL of 2.0 M H_2O_2 solution, The reaction mixture was incubated at ambient conditions for about 0.5 hours and then the oxidation reaction was terminated by addition of sulfuric acid into the reaction media. Afterward, the spectrum of the colored product was recorded and the λ_{max} at 450.0 nm of the resulted TMB dimine was utilized for calculating the enzyme activity in μM min^{-1}, considering that the molar absorption coefficient of TMB dimine at 450.0 nm is about 5.9×10^4 mol^{-1} cm^{-1}. It is mentionable that the relative and residual nanozymatic activity of the as-prepared nanocomposite was calculated by the following formulas [41,42];

$$\text{Residual activity (\%)} = (\text{Activity}/\text{Activity of control}) \times 100$$

$$\text{Relative activity (\%)} = (\text{Activity}/\text{Maximal activity}) \times 100$$

3. Results and discussion

3.1. pH stability of SiO_2@Au nanocomposite

The pH effect on the enzyme-like activity of the as-prepared SiO_2@AuNPs nanocomposite was evaluated by measuring its relative activity over a pH range of 3.0-7.0. The above-mentioned studies were performed to provide insight into the stability of the enzyme-like the as-prepared SiO_2@AuNPs nanocomposite against environmental pH changes (Figure 1). The results shown in this figure exhibited that the as-prepared SiO_2@AuNPs nanocomposite show its maximum enzyme-like activity at pH =4.0 and then its activity was slightly decreased by increasing the pH of media, reaching 83.3%

at pH=5.0. The activity of the as-prepared SiO_2@AuNPs nanocomposite reached 6.6% of its maximum value at pH=7.0.

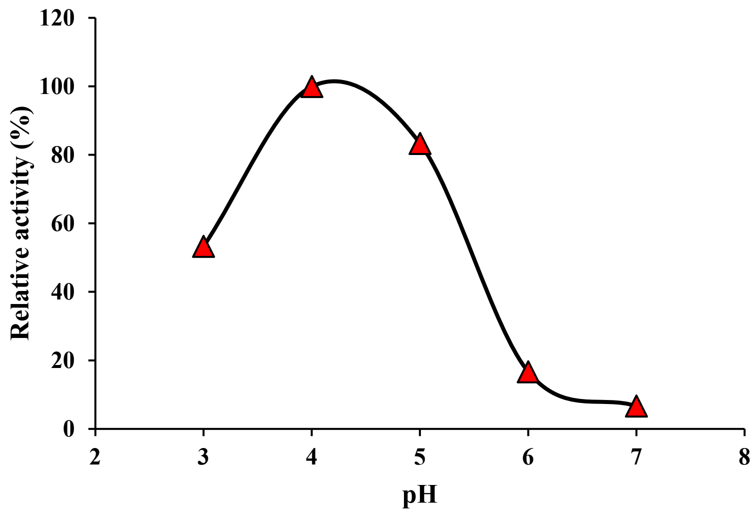

Figure 1. The effect of pH on the enzyme-like activity of the as-prepared SiO_2@AuNPs nanocomposite.

3.2. Cycling stability of SiO_2@Au nanocomposite

To evaluate the cycling stability of the as-prepared SiO_2@AuNPs nanocomposite, the reusability studies were performed as a reliable way for proving the recoverability and cycling stability of nanocatalysts [5]. To do this, the activity of the as-prepared nanocomposite in the first operational time was considered 100% and used as the control for calculating the residual activity of nanocomposite in successive operational cycles. The plot of the residual activity of the nanocomposite as a function of operational times was shown in Figure 2, as shown in this figure, the as-prepared nanozymes can save their initial activity during 4 operational cycles. After that, their activity slightly decreased and reached 90% of their initial activity at the 5th operational cycle. Considering these results, it can be concluded that the as-prepared SiO_2@AuNPs nanocomposite can be utilized as a high-throughput nanozymes with excellent reusability and cycling stability.

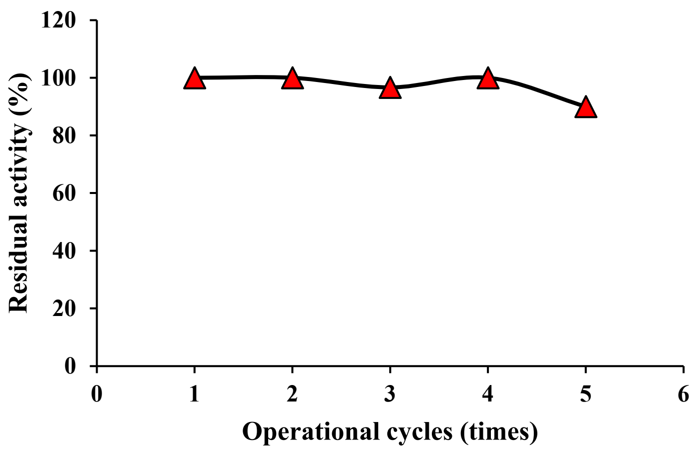

Figure 2. Reusability of the as-prepared SiO_2@AuNPs nanocomposite as peroxidase mimics.

3.3. Storage stability of SiO_2@Au nanocomposite

The shelf-life (shelf stability) of the as-prepared SiO_2@AuNPs nanocomposite was investigated upon their storage in acetate buffer (pH=4.0) at ambient conditions for about one month. The activity of the as-prepared nanocomposite on the first day of storage was considered 100% and used as the control for calculating the residual activity of the nanocomposite. Thereafter, the residual activity of the nanozymes was determined in different time intervals during the storage as an index for estimating their shelf stability. The plot of residual activity of the nanocomposite as a function of storage time (day) was shown in Figure 3, revealing that the as-prepared SiO_2@AuNPs nanocomposite save 100% of their initial activity after 30 days of storage at ambient conditions. Considering these results, it can be concluded that the as-synthesized SiO_2@AuNPs nanocomposite can be used as excellent enzyme alternatives with high shelf stability as a significant advantage from a practical point of view.

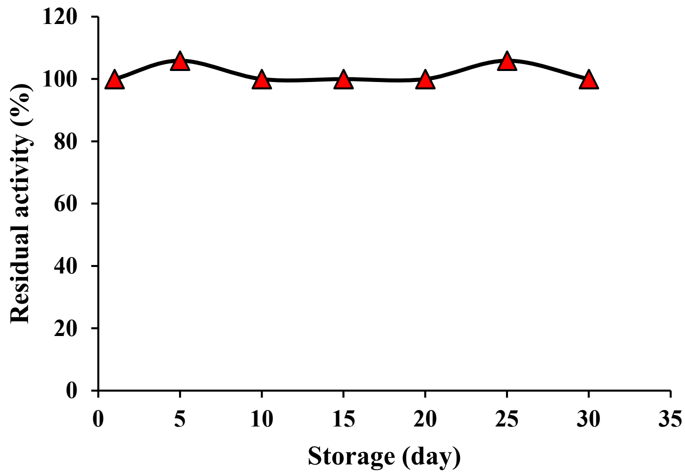

Figure 3. Shelf-life of the as-prepared SiO_2@AuNPs nanocomposite as peroxidase mimics.

3.4. Kinetic performances of SiO_2@AuNPs nanocomposite

Kinetic studies were carried out to calculate the kinetic parameters of the as-prepared SiO_2@AuNPs nanocomposite toward 2-electron reversible oxidation of TMB. In fact, the kinetic parameters of an enzyme were previously well defined with numeric values including affinity constant (K_m) and maximum enzymatic velocity (V_{max}) utilizing the Michaelis–Menten steady-state kinetics model. The V_{max} value reflects the intrinsic properties of an enzyme or nanozyme and is defined as the highest possible rate of the nanozyme-catalyzed reaction when all enzyme molecules or all nanozyme particles are saturated with the substrate which pointed to the catalytic efficiency of an enzyme or nanozyme. Hence, the higher value of V_{max} for an enzymatic/nanozymatic reaction can be assigned to the higher catalytic efficiency of the enzyme or nanozyme [40–42]. In contrast, the affinity of the substrate of an enzyme or nanozyme for interaction with its active site is represented by the K_m, as reported [40]. In fact, the lower values of K_m pointed to the higher affinity of the substrate for binding to the enzyme/nanozyme active site/nodes [17,40]. Therefore, to quantify the kinetics parameters of the as-prepared SiO_2@AuNPs nanocomposite, the Michaelis–Menten saturation curve was obtained via plotting the nanozymatic reaction velocity as a function of the substrate (TMB) concertation (Figure 4A). As seen in Figure 6A, the reaction rate of the SiO_2@AuNPs nanocomposite-mediated oxidation reaction was increased by increasing the TMB concertation and then reaching a steady-state condition. For accurate estimation of K_m and V_{max} of the SiO_2@AuNPs nanocomposite-mediated oxidation reaction, the Lineweaver–Burk plot was also constructed for the SiO_2@AuNPs nanocomposite-mediated reaction (Figure 4B). The results revealed a V_{max} of 1.35 μM min^{-1} and a K_m as very low as 0.06 mM for the SiO_2@AuNPs nanocomposite. Considering the low K_m value of the as-synthesized nanocomposite, it can be concluded that the as-prepared nanocomposite shows high substrate affinity, as reported [17,41]. Besides, obtaining a V_{max} of 1.35 µM min^{-1}, revealed that the as-prepared nanocomposite has a very good catalytic efficiency.

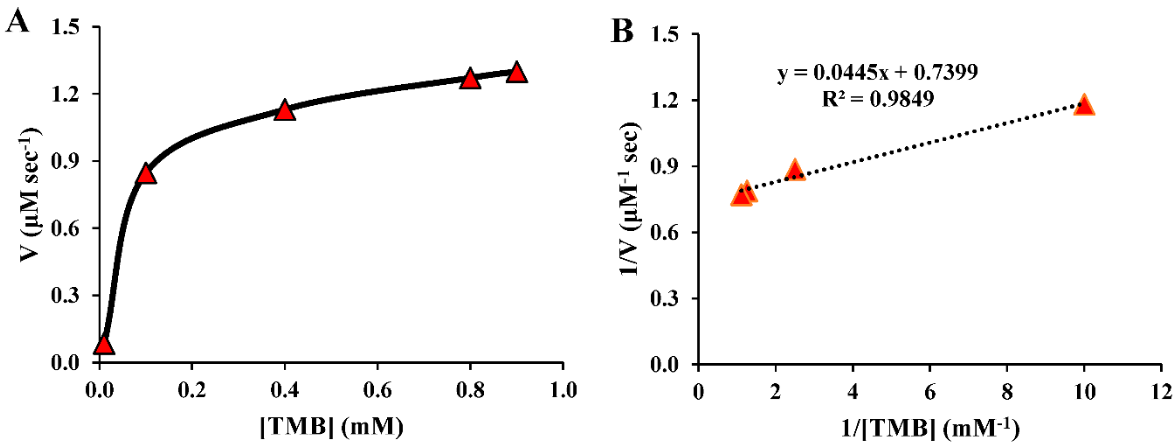

Figure 4. (A) Michaelis–Menten curve and (B) Lineweaver–Burk plot for the as-prepared SiO_2@AuNPs nanocomposite as peroxidase mimics.

4. Conclusions

In this study, considering the peroxidase-like activity of the SiO_2@AuNPs nanocomposite, their biochemical properties including, pH stability, cycling stability, shelf stability, and kinetic parameters were investigated. The results revealed that the as-mentioned SiO_2@AuNPs nanocomposite reveal its maximal activity at pH=4.0 along with saving 83.3% of its maximal activity at pH=5.0. Besides, the reusability and shelf-storage studies exhibited that the SiO_2@AuNPs nanocomposite retained 90% and 100% of its initial activity after 5 operational cycles and 30 days of storage, in order. The kinetic parameters of the as-prepared nanozymes were calculated using Menten kinetic model, revealing a V_{max} of 1.35 µM min^{-1} and a K_m as low as 0.06 mM for the SiO_2@AuNPs nanocomposite. Based on the results of this work, the as-prepared SiO_2@AuNPs nanocomposite with intrinsic peroxidase-like activity shows high substrate affinity and catalytic efficiency along with excellent cycling- and shelf-stability, making them suitable for application in peroxidase-mediated reactions instead of the native enzyme.

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Conflict of interest: None.

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