ISSN: 2639-7536

Research Article

Petroleum and Chemical Industry International

Evaluation of Biochemical Behavior and Stability of Gold Nanoparticles with High Intrinsic Peroxidase-Like Activity

Saeed Reza Hormozi Jangi *

Hormozi Laboratory of Chemistry and Biochemistry, Zabul, Iran

***Corresponding Author**
Saeed Reza Hormozi Jangi, Hormozi Laboratory of Chemistry and Biochemistry, Zabul, Iran.

Submitted: 2023, July 01; **Accepted**: 2023, July 26: **Published**: 2023, Aug 02

Citation: Hormozi Jangi, S. R. (2023). Evaluation of Biochemical Behavior and Stability of Gold Nanoparticles with High Intrinsic Peroxidase-Like Activity. *Petro Chem Indus Intern, 6*(4), 234-239.

Abstract

In this study, the biochemical behavior and stability of gold nanoparticles with intrinsic peroxidase-like activity were evaluated as potential native enzyme alternatives. The gold nanoparticles were synthesized at physiological temperature using bovine serum albumin as the stabilizer and then characterized by the TEM imaging method. Afterward, their peroxidase-like activity was checked upon irreversible oxidation of 3,3'-diaminobenzidine to produce a brown-colored indamine polymer, and the specific enzyme-like activity of the as-prepared nanoparticles was also calculated. The results showed a specific activity as high as 0.4212 UI µM-1 for the as-prepared gold nanoparticles. Thereafter, their stability and biochemical performances were evaluated considering their enzyme-like activity as a reliable index. The as-prepared nanoparticles showed their maximal activity at pH=5.0 and 20.0±1.0 ℃ according to the results of pH and thermal stability studies, in order. Besides, the nanoparticles saved above 80.0% of their maximal activity over pH=3.0-4.0. As a significant advantage compared to the natural enzymes, the as-synthesized gold nanoparticles revealed a pH-independent enzyme-like activity over a wide pH range of pH=7.0-10.0 along with a temperature-independent activity over t=23-28℃. The salt stability studies showed that their activity was not affected by variations in the ionic strength of the reaction media. The kinetics results showed a Vmax of 83.3 µM min-1 and a Km as very low as 0.005 M for the gold nanoparticles. Considering the above results, the as-prepared gold nanoparticles can be considered high stable nanozymes with high intrinsic peroxidase-like activity and excellent catalytic efficiency.

Keywords: Gold Nanoparticles, Intrinsic Peroxidase-Like Activity, Enzyme-Like Activity, Biochemical Stability, Specific Activity.

1. Introduction

Concerning the fast and great development of nanotechnology as well as considering the significant applications of nanomaterials in modern life, the research on the development of novel nanomaterials and the design of new economical synthesis methods for important nanomaterials was attracted the attention of scientists and researchers [1, 2]. In this regard, several novel nanoscale materials were synthesized and characterized by researchers, for instance, noble-metal-based nanomaterials, carbon dots, quantum dots, metal-organic frameworks, magnetic nanoparticles [1-11] etc. Besides, the synthesis of novel materials, scientists focused on the evaluation of potential properties of the nanomaterials such as catalytic properties, optical properties, anti-cancer features anti-bacterial characteristics biocompatibility and enzyme-like properties [6,7,12-18]. The importance of these types of research can be understandable when a nonmaterial needs to be practically or commercially used for a real application, for instance, using a nanomaterial as a nanodrug toward cancer treatment or application for water safety considerations [19-22]. Among various nanoparticles with different properties, recently, nanomaterials with enzyme-like properties were introduced as enzyme alternatives for catalyzing industrial, clinical, and environmental enzyme-mediated reactions at harsh conditions with lower cost and higher efficiency along with recyclability [23, 24]. In this regard, several nanoparticles with peroxidase-like activity such as silver nanoparticles , MnO_2 nanoparticles, metal-organic frameworks , and silica-coated-Fe_3O_4 nanoparticles were introduced after the first report of nanozymes in 2007 [22,25-28]. Besides, recently, the excellent peroxidase-like activity of gold-based nanozymes attracted good attention for application as alternatives to natural peroxidase toward different applications in sensing, biosensing, organic dye degradation, and catalysis [2,29-31]. However, despite the wide potential application of gold-based nanozymes as enzyme alternatives, unfortunately, up to now, there is no report on their biochemical behavior and stability. Hence, in this study, the biochemical behavior and stability of gold nanoparticles with intrinsic peroxidase-like activity were evaluated considering their enzyme-like activity as an index for estimation of their stability and biochemical performances. In this regard, gold nanoparticles were synthesized at physiological temperature using bovine serum

albumin as the stabilizer and then characterized by the TEM imaging method. Afterward, their peroxidase-like activity was checked upon irreversible oxidation of 3,3'-diaminobenzidine to produce a brown-colored indamine polymer, and the specific enzyme-like activity of the as-prepared nanoparticles was also calculated. Besides, their biochemical stability was also checked by evaluating the pH stability, thermal stability, and salt stability of the as-prepared nanoparticles. Moreover, kinetics studies were performed to investigate the catalytic efficiency and substrate affinity of the as-mentioned gold nanoparticles. Considering the obtained results, the as-prepared gold nanoparticles can be considered high stable nanozymes with high intrinsic peroxidase-like activity and excellent catalytic efficiency.

2. Experimental

2.1. Materials and Instrumentations

NaCl, hydrogen peroxide, HAuCl4.4H2O, bovine serum albumin, and NaOH from Merck, 3,3'-diaminobenzidine and phosphoric acid from Sigma Aldrich, and deionized water from Zolal Teb Shimico (Iran) company were obtained. A Chrom Tech UV–Vis spectrophotometer (model: UV 3300) was utilized for nanozyme activity assay, stability studies, and kinetics evaluation. Besides, the TEM micrographs of the as-prepared gold nanoparticles were recorded using a transmission electron microscope (Zeiss, model EL10C) operated at an accelerating voltage of 80 kV.

2.2. Synthesis of Gold Nanoparticles With Intrinsic Peroxidase-Like Activity

To synthesize the gold nanoparticles, 10.0 mM HAuCl4.4H2O (5.0 mL) was introduced to 50 mg mL^{-1} bovine serum albumin (5.0 mL), followed by stirring at 37 °C and adding 1.0 M NaOH to adjust pH. The solution was incubated at 37 °C for 12 hours to complete the synthesis process.

2.3. Nanozyme Activity Assay

In a typical test, 80.0 µL of gold nanoparticles, 200.0 µL DAB (with a final concentration of 0.245 mM), and 40.0 µL of 30% HP were introduced into 1300 µL of 0.4 M phosphate buffer (pH 7.0), followed by ambient mixing for 25.0 min. After that, the absorbance of the brown-colored product was recorded at 460.0 nm considering a molecular extinction coefficient ε=5500 molar cm-1. Notably, the nanozyme relative activity was calculated using the following formula [32];

Relative activity= (activity/maximal activity)×100

3. Results and Discussion

3.1. Characterization of As-Prepared Gold Nanoparticles

The as-prepared gold nanoparticles were characterized by the TEM imaging method. The results shown in Figure 1 revealed that the as-prepared nanoparticles are approximately uniform and small in size with a size distribution over 4.6-37.3 nm and a mean size of 12.4 nm (n=21). To obtain a better view of the size distribution of the as-prepared nanoparticles, the histogram of the particle size as a function of frequency was provided (inset of Figure 1), as can be seen from this histogram, the as-prepared nanoparticles have a median size as small as 13.9 nm with a narrow size distribution which makes them suitable for application in nanozyme-based systems because the multi-size distributed nanoparticles commonly show lower peroxidase-like activity than the nanoparticles with a narrow size distribution, as reported [29].

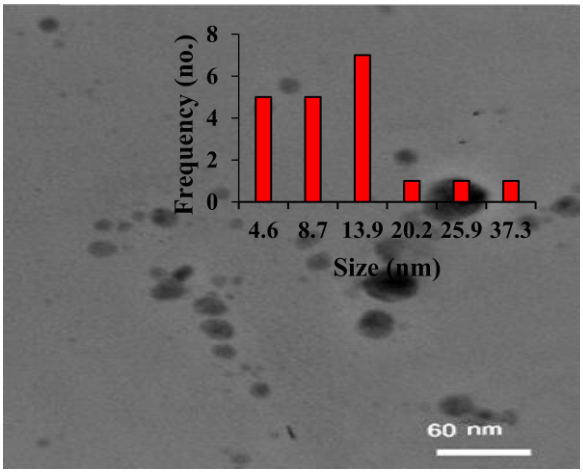

Figure 1: TEM image of the as-synthesized gold nanoparticles, inset: histogram of the particle size as a function of frequency.

3.2. Investigation of Peroxidase-Like Activity

The applicability of the as-prepared nanoparticles toward potential application in nanozyme-based catalysis was investigated by evaluation of their peroxidase-like activity via spectrophotometric probing the oxidation process of DAB with hydrogen peroxide in the presence of the as-prepared nanoparticles. In this regard, the brown-colored oxidation product of DAB (polyDAB) with maximal absorbance at 460.0 nm was selected as an analytical probe system to quantify the activity of the as-prepared nanoparticles for the peroxidase-mediated oxidation process of DAB. The results are shown in Figure 2, as shown in this figure, the DAB oxidation in the absence of the as-prepared nanoparticles cannot proceed and the absorbance at 460.0 nm cannot be observed. In contrast, by introducing the as-prepared nanoparticle in the reaction media, the oxidation was catalyzed and the characteristic absorbance of polyDAB at 460.0 nm appeared, revealing the successful catalyzing of the process by the as-prepared nanoparticles. It is mentionable that initially, the as-prepared gold nanoparticles act on the hydrogen peroxide to produce the active hydroxyl radical, then the produced hydroxyl radicals react with DAB via a 2-electron process to produce a DAB cation (DAB^{+}), the reaction was followed by interacting the produced DAB+ with a DAB molecule, resulting in a DAB dimer ($(DAB)_2$). The above-mentioned cycle was repeated to produce the final polymeric product of the oxidation process with a considerable absorbance at 460.0 nm [26, 27,29,30]. Considering these results, it can be concluded that the as-prepared nanoparticles show significant peroxidase-like activity and can be used in enzyme-mediated catalytic processes instead of the natural peroxidase enzymes.

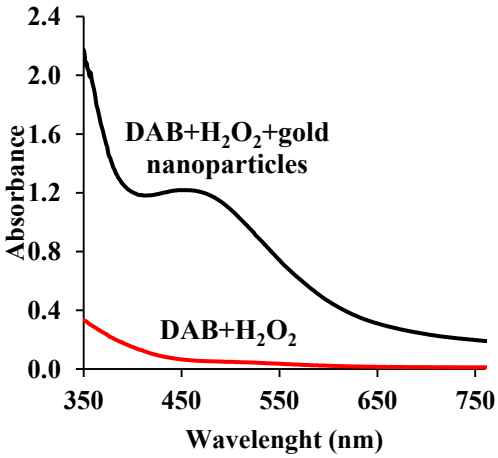

Figure 2: Evaluation of the peroxidase-like activity of the as-prepared gold nanoparticles

3.3. Calculating Specific Enzyme-Like Activity

To explore more precise on the catalytic activity of the as-prepared nanoparticles, it is necessary to quantify their specific enzyme-like activity. To calculate the specific enzyme-like activity, the plot of nanozymatic activity (in μM min^{-1} =UI) as a function of nanoparticle amount (μM) was constructed and the nanoparticles' enzyme-like specific activity was estimated from its slope. The results are shown in Figure 3, revealing a specific enzyme-like activity as high as 0.4212 μM min^{-1} μM^{-1} nano or simply 0.4212 UI μM^{-1}nano. Considering these results, the as-prepared nanoparticles show very high peroxidase-like activity with a high specific enzyme-like activity which makes it appropriate for its application as an enzyme alternative in enzyme-catalyzed reactions.

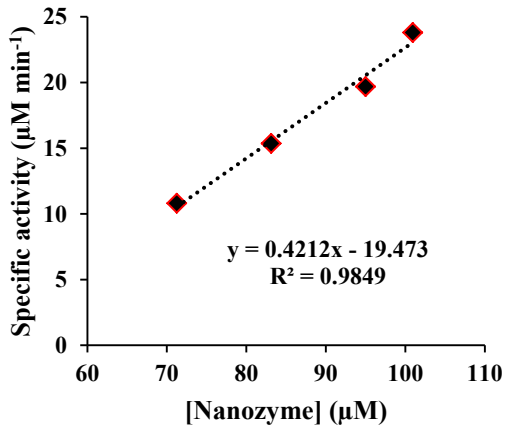

Figure 3: Quantification of the specific enzyme-like activity of the as-prepared gold nanoparticles.

3.4. pH Stability

The pH effect on the enzyme-like activity of the as-prepared gold nanoparticles was evaluated by measuring their relative activity over a pH range of 3.0-12. The above-mentioned studies were performed to provide insight into the stability of the enzyme-like nanoparticles against environmental pH changes (Figure 4). The results shown in Figure 4 revealed a maximum enzyme-like activity at pH =5.0 for the as-prepared gold nanoparticles. It should be noted that the as-synthesized enzyme-like nanomaterials saved about 80.0% of their maximal activity over pH=3.0-4.0. Notably, As a significant advantage compared to the natural enzymes, the as-synthesized gold nanoparticles revealed a pH-independent enzyme-like activity over a wide pH range of pH=7.0-10.0.

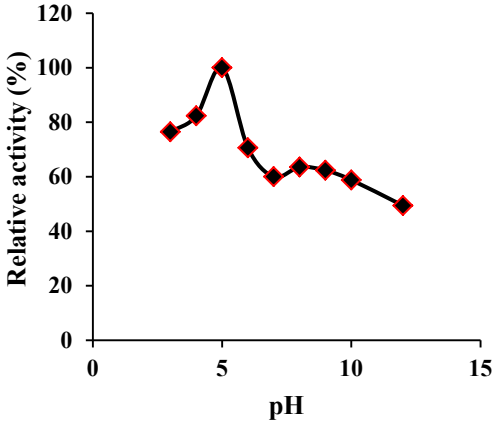

Figure 4: The effect of pH on the enzyme-like activity of the as-prepared gold nanoparticles

3.5. Thermal Stability

The thermal stability of the as-mentioned gold nanoparticles was investigated by calculating the relative activity of the as-prepared enzyme-like nanoparticles over a temperature range of 18±1.0 -28±1.0 ℃. The results shown in Figure 5 exhibited a maximum enzyme-like activity for the as-prepared nanoparticles at 20.0±1.0 ℃. As a significant advantage compared to the natural enzymes, the as-synthesized gold nanoparticles revealed a temperature-independent activity over a wide temperature range as wide as 23±1.0 -28±1.0 ℃, showing their high thermal stability.

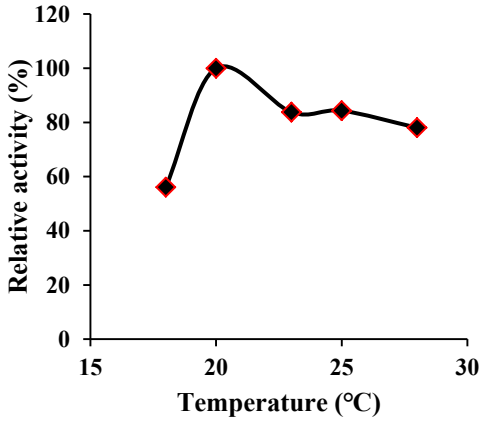

Figure 5: The effect of temperature on the enzyme-like activity of the as-prepared gold nanoparticles

3.6. Salt Stability

The stability of the as-mentioned gold nanoparticles against high salt concentrations as a serious problem of the native enzymes was investigated over 5-500 mM of NaCl as a model salt. To probe

the salt stability, the enzyme-like activity of the as-mentioned gold nanoparticles was calculated after exposure to high salt concentration. The results of this study are shown in Figure 6, revealing that the as-mentioned gold nanoparticles can save their maximal activity over a salt concentration range as wide as 5-500 mM. Based on the above results it can be concluded that the 5-500 mM can be used for catalyzing the peroxidase-mediated oxidation reactions at high salt concentrations without any decrease in catalytic efficiency and nanozymatic activity instead of the unstable native peroxidase.

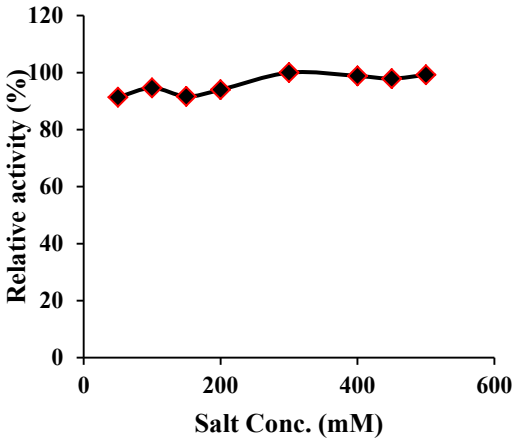

Figure 6: The salt stability results for the as-mentioned gold nanoparticles

3.7 Calculating the Kinetic Factors

Kinetic studies were carried out to estimate the kinetic parameters (i.e., K_m and V_{max}) of the as-prepared MnO_2 nanozyme as pseudo-peroxidase nano enzyme toward n-electron irreversible oxidation of 3,3'-diaminobezedine. It is well known that the V_{max} value reflects the intrinsic properties of the enzyme/nanozyme and is defined as the highest possible rate of the nanozyme-catalyzed reaction (i.e., catalytic efficiency) when all enzyme molecules or all nanozyme particles are saturated with the substrate. The higher value of V_{max} is assigned to the higher catalytic efficiency of the enzyme/nanozyme. In contrast, the affinity of the substrate of an enzyme/nanozyme to interact with its active site is represented by the K_m value, the lower values indicate a higher affinity of the substrate for binding to the enzyme/nanozyme. To evaluate the kinetics performances of the as-prepared gold-nanozymes, the Michaelis–Menten plot was constructed by plotting the velocity of the nanozymatic reaction as a function of DAB concertation. The results are shown in Figure 7. As seen in Figure 7A, the rate of gold-nanozyme-mediated oxidation reaction was increased by increasing the substrate concertation and then leveling off. Besides, to explore more precise on the kinetic performances of gold-nanozymes toward DAB oxidation, the Lineweaver–Burk plot was also constructed for gold-nanozymes mediated reaction for accurate estimation of Km and Vmax of the gold enzymes-mediated oxidation reaction. The results shown in Figure 7B revealed a V_{max} of 83.3 µM min^{-1} and a Km as very low as 0.005 M for the gold nanoparticles.

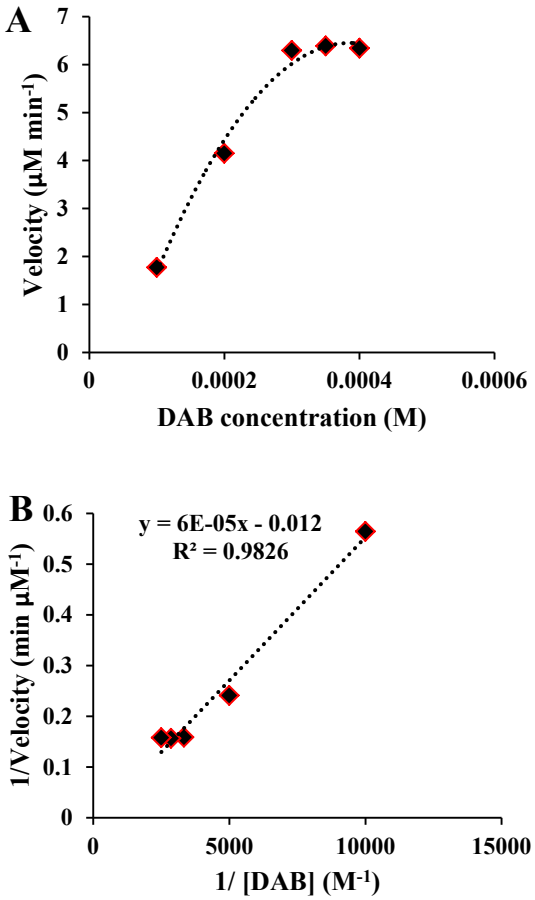

Figure 7: (A) Michaelis–Menten plot and (B) Lineweaver-Burk linear plot for gold-nanozymes mediated reaction.

4. Conclusions

In this study, the biochemical behavior and stability of gold nanoparticles with intrinsic peroxidase-like activity were evaluated. The gold nanoparticles were synthesized at physiological temperature using bovine serum albumin as the stabilizer and then characterized by the TEM imaging method. Afterward, their peroxidase-like activity was checked upon irreversible oxidation of 3,3'-diaminobenzidine to produce a brown-colored indamine polymer, and the specific enzyme-like activity of the as-prepared nanoparticles was also calculated. The results showed a specific activity as high as 0.4212 UI μM^{-1} for the as-prepared gold nanoparticles. The as-prepared nanoparticles showed their maximal activity at pH=5.0 and 20.0±1.0 ℃ according to the results of pH and thermal stability studies, in order. Besides, the nanoparticles saved above 80.0% of their maximal activity over pH=3.0-4.0 and t=23-28℃ along with a constant activity over a pH range of 7.0-10.0. The salt stability studies showed that their activity was not affected by variation in the ionic strength of the reaction media.

The kinetics results showed a Vmax of 83.3 μM min-1 and a Km as very low as 0.005 M for the gold nanoparticles. Considering the above results, the as-prepared gold nanoparticles can be considered high stable nanozymes with high intrinsic peroxidase-like activity and excellent catalytic efficiency.

Acknowledgments

The authors gratefully thank the Hormozi Laboratory of Chemistry and Biochemistry (Zabol, Iran) for the support of this work.

Conflict of interest

None.

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