Article

Evaluating the Effect of Shelf-Storage, Daylight, and Air Oxygen on the Peroxidase-like Activity of Unmodified Silver Nanoparticles

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Abstract: In this contribution, unmodified silver nanoparticles were synthesized and characterized for their size and morphological properties. Thereafter, their peroxidase-like activity as the common catalytic property of silver nanoparticles was investigated by catalyzing the oxidation of 3,3′,5,5′-tetramethyl-benzidine (TMB) as peroxidase substrate, exhibiting, a specific activity as high as 5.4 μM min^{-1} for the as-prepared unmodified silver nanoparticles. The stability of the catalytic activity of the as-prepared nanozymes was also checked upon their storage at ambient temperature within 7 days at different storage conditions. The results revealed that the peroxidase-like activity of unmodified silver nanoparticles was approximately retained at about 75%, and 63% after 7 days exposing daylight and air oxygen, in order. The shelf-life (storage stability) of the as-prepared nanozymes was also investigated at usual storage conditions (i.e., 4 °C under dark), revealed that the nanozymes saved their activity about 96% of their initial activity after 10 days of storage at 4 °C under dark conditions.

Keywords: unmodified silver nanoparticles; peroxidase-like activity; air oxygen; daylight; shelf-life

Introduction

Nowadays, metal-based nanoparticles, especially silver nanoparticles (AgNPs) have been widely used in different research fields due to their excellent optical, anti-cancer, and anti-bacterial properties along with biocompatibility [1]. Especially, the fast development of nanoscience and material chemistry caused an enhanced interest in the research on the synthesis and characterization of novel nanomaterials via new methods for achieving the nano-compounds with unique catalytic activity [1,2], characteristic optical properties [3], and excellent medicinal properties [4] along with high biocompatibility [5]. In fact, the mission of nanobiotechnology as one of the most attractive fields of nanotechnology [6,7] is the synthesis and characterization of these nanomaterials using different and green approaches.

Among different nanomaterials, metal nanoparticles have been used for the construction of a wide variety of nanosensors and biosensors for the determination of several analytes such as explosives [8], heavy metals [9], and biomaterials [10]. However, their application in medical science was also damned, especially for the design of hematological tests to diagnose different diseases for instance neurodegenerative diseases [11]. In addition, recently, employing the catalytic activity of these nanoparticles for practical applications was also attracted several researchers [12,13]. The new field of catalysis which was introduced as an alternative to enzyme-based catalysis is called enzyme-based catalysis. Nanozymes are defined as nanomaterials with high enzyme-like activity and can be used for the simulation of enzymatic reactions in extreme environmental conditions [12,14–16]. In fact, natural enzymes show several disadvantages as follows [17]; (I) low stability (thermal and narrow pH range) (II) difficulty in recovery, and (III) no reusability of the enzyme. Commonly, for overcoming these drawbacks, the enzyme immobilization process has been developed [18–20].

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Another approach for overcome to these difficulties is utilizing the high stable nanozymes with high enzyme-like activity in the enzyme-catalyzed reactions [17]. Among different nanomaterials with enzyme-like activity, noble metal nanoparticles are considered excellent alternatives for the enzymes due to their high enzyme-like activity, high stability, and unique green properties [11,14]. Regarding these nanozymes, silver nanoparticles had been used in different research fields due to their inexpensive simple preparation routes, biocompatibility, and excellent optical and high semi-peroxidase properties [2]. However, it is well-known that the optical properties of unmodified silver nanoparticles are extremely sensitive to environmental conditions (e.g., light, air oxygen, etc.), hence, commonly, silver nanoparticles need to be modified and stabilized by stabilizers (e.g., biopolymers, biological stabilizers, etc.) to save their optical features and makes them suitable for practical applications. In this regard, the biosynthesis of AgNPs, biological materials such as microalgae extract [21], chitosan [22], Artemisia 'scoparia extract [23], and Laurencia caspica macroalgae [24] have been used as both stabilizers for surviving silver nanoparticles from the significant decrease of optical absorbance during their storage via enhancing their stability against environmental conditions.

Based on our best knowledge, the scientific information about the stability of the catalytic properties of unmodified silver nanoparticles against environmental conditions such as light and air oxygen is limited. Hence, in this contribution, unmodified silver nanoparticles were synthesized and characterized for their size and morphological properties. Thereafter, their peroxidase-like activity as the common catalytic property of silver nanoparticles was investigated. Afterward, the stability of the catalytic activity of the as-prepared nanozymes was also checked upon their storage at ambient temperature in different storage conditions dark, daylight, and open air.

Experimental Section

2.1. Materials and instrumentations

All materials were obtained from Merck Company in their analytical grade. The UV-Visible spectra were recorded by an Ultrospec 4000 UV-Vis spectrophotometer manufactured by Pharmacia Biotech (Biochrom) Ltd. equipped with SWIFT Software. A Metrohm 827 pH lab pH meter equipped with a combined glass electrode was used for pH measuring for buffer preparation. TEM micrograph of the as-prepared nanozymes was done by a transmission electron microscope (Zeiss, model EL10C) operated at an accelerating voltage of 80 kV.

2.2. Synthesis of unmodified silver nanoparticles

Silver nanoparticles were synthesized based on the literature [1]. Briefly, 5.0 mL of 10.0 mM $AgNO_3$ was mixed with 5.0 mL sodium citrate (10.0 mM). After that, 89.0 mL DI water was added to the mixture, and the resulting solution was mixed for 20 min at room temperature. The synthesis process was followed by the quick addition of $NaBH_4$ (8.8 mg) and stirring for 2.0 hours at the ambient temperature. Finally, yellow-colored silver nanoparticles were stored at 4 ºC under dark conditions for future uses.

2.3. Evaluating peroxidase-like activity

To evaluate the peroxidase-like activity of the as-prepared nanozymes, 20 µL hydrogen peroxide solution (final concentrations of 10.0 µM, 50 µM, and 80.0 µM), and 50 µL of TMB (final concentration in the reaction solution, 0.4 mM), and 80 µL of unmodified silver nanozymes were added to 1.0 mL of acetate buffer (0.3 M; pH, 0.4). Then, to complete the substrate (TMB) oxidation process, the reaction solution was incubated for about 10 minutes at ambient temperature. After that, the absorbance of the oxidation product (blue-colored) was recorded at 658 nm [2]. The specific activity of nanozymes (µM sec^{-1}) was then calculated using the absorbance coefficient of the oxidation product at 658 nm (ε=39000). Notably, the relative and residual activity of nanozymes were calculated by the following formulas [18];

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$$Residual\ activty = \frac{Activity}{Activty\ of\ control} \times 100$$

3. Results and discussion

3.1. Characterization of nanozymes

Unmodified silver nanoparticles were synthesized and characterized for their size and morphological properties. In this regard, the TEM image of the as-prepared nanozyme was recorded and the results are shown in Figure 1, as shown in this figure, the as-prepared silver nanoparticles showed uniform morphology with spherical particles. In addition, the as-prepared nanozymes showed a narrow size distribution over 10.3-12.6 nm with an average size of 11.0 nm.

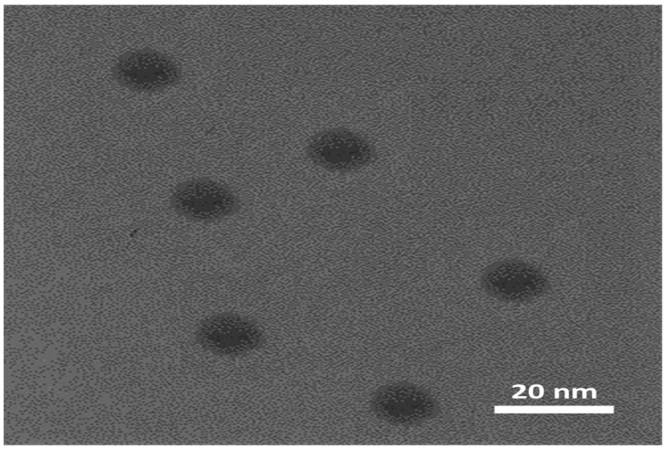

Figure 1. TEM image of as-prepared unmodified silver nanoparticles.

3.2. Evaluating peroxidase-like activity of as-prepared nanozymes

The peroxidase-like activity of the as-synthesized nanoparticles was investigated using 3,3′,5,5′-tetramethylbenzidine (TMB) as the peroxidase substrate and its blue-colored oxidation product (i.e., TMB-ox) was utilized as an analytical probe for quantification of nanozyme activity. The results are shown in Figure 2, as shown in this figure, in the presence of TMB, the as-synthesized nanozymes catalyze the oxidation process of TMB by hydrogen peroxide to produce its corresponding blue-colored cation radical, TMB-ox with a shoulder 440-485 nm (λ_{max} of 460 nm) and a symmetric spectrum over 500-750 nm (λ_{max} of 658 nm). In fact, during the oxidation of TMB silver nanozymes produce active hydroxyl radicals by acting on hydrogen peroxide [2,8,10–12]. The produced radicals then oxidize the TMB molecules to their corresponding cation radicals via a 2-electron reversible oxidation pathway. It should be mentioned that the specific activity of the as-prepared nanozymes was calculated at about 1.36 μM min^{-1}, 3.66 μM min^{-1}, and 5.4 μM min^{-1} for 10.0 μM, 50.0 μM, and 80.0 μM of hydrogen peroxide as the active agent, in order, in the presence of a constant concentration of TMB (enzyme substrate).

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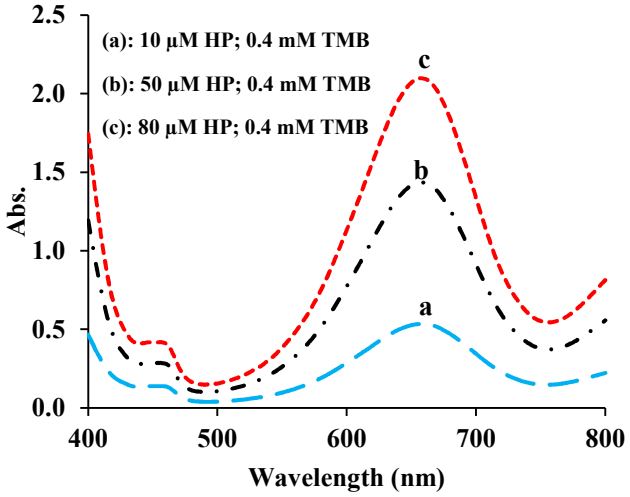

Figure 2. Evaluation of the peroxidase-like activity of the as-prepared unmodified silver nanoparticles.

3.3. Effect of daylight on peroxidase-like activity of as-prepared nanozymes

The effect of daylight on the peroxidase-like activity of as-prepared unmodified silver nanoparticles was evaluated by exposing them to daylight upon storage at ambient temperature for 7 days. The activity of the as-prepared nanozymes on 1st day was used as the control and considered 100%, then the residual activity of the nanozymes was day-by-day estimated against this control to investigate their stability upon exposure the daylight. The results shown in Figure 3 reveal that the peroxidase-like activity of the as-prepared nanozymes was decreased after exposing daylight and reached about 75% after 7 days of storage. This reduction of activity can be contributed to particle aggregation of nanoparticles by light. The aggregation of the nanoparticles leads to an increase in their size and consequently, their catalytic performances will reduce. Besides, daylight can catalyze the surface oxidation of these nanoparticles which cause to reduce their catalytic activity.

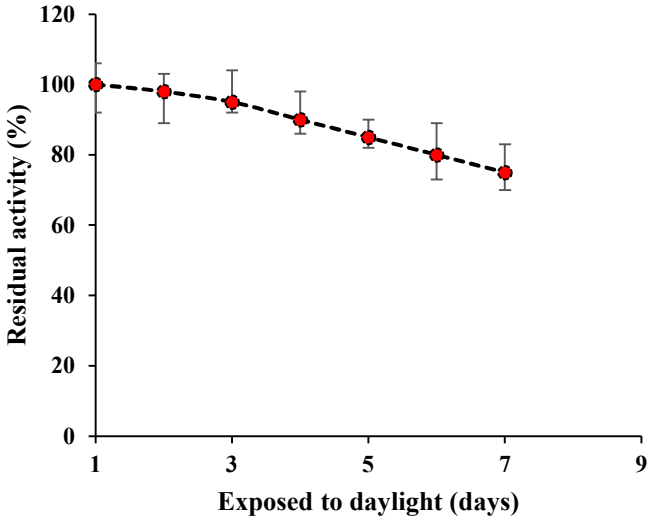

Figure 3. The effect of daylight on peroxidase-like activity of as-prepared nanozymes.

3.4. Effect of air oxygen on peroxidase-like activity of as-prepared nanozymes

In the open air, the air oxygen can affect the silver nanoparticles, proceed with the surface oxidation processes, and consequently reduce the catalytic activity of these nanozymes. Hence, the effect of air oxygen on the peroxidase-like activity of the as-prepared unmodified silver nanoparticles

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was evaluated by their storage in the open air at ambient temperature for 7 days. The activity of the as-prepared nanozymes on 1st day was used as the control and considered 100%, then the residual activity of the nanozymes was day-by-day calculated to investigate their stability upon exposure to the air oxygen. Notably, for exploring more precisely the accuracy of the results, the nanozymes solutions were covered by foil (dark conditions) to eliminate the effect of daylight on their activity. The results shown in Figure 4 revealed that the peroxidase-like activity of the as-prepared nanozymes was decreased after exposure to air oxygen and reached about 63% after 7 days of storage in the open air.

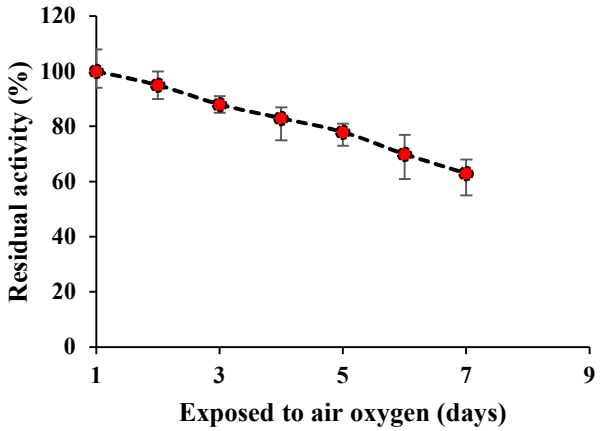

Figure 4. The effect of air oxygen on peroxidase-like activity of as-prepared nanozymes.

3.5. Storage stability of as-prepared nanozymes

The shelf-life (storage stability) of the as-prepared nanozymes was investigated at usual storage conditions of silver nanoparticles (i.e., 4 ℃ under dark). The results are shown in Figure 5, as shown in this figure, the as-prepared nanozymes saved about 96% of their initial activity after 10 days of storage at 4 ℃ under dark conditions. Considering these results, it can be concluded that upon suitable storage conditions, the unmodified silver nanoparticles can be used as excellent enzyme alternatives for proceeding enzyme-catalyzed reactions with high enzyme-like activity and very good shelf-life.

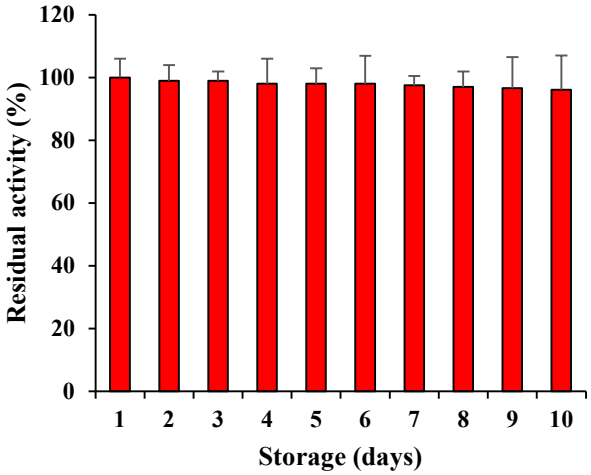

Figure 5. Shelf-life of the as-prepared nanozymes.

4. Conclusions

In this contribution, unmodified silver nanoparticles were synthesized and characterized for their size and morphological properties. Thereafter, their peroxidase-like activity as the common

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catalytic property of silver nanoparticles was investigated by catalyzing the oxidation of 3,3′,5,5′-tetramethyl-benzidine (TMB) as peroxidase substrate, exhibiting, a specific activity as high as 5.4 μM min^{-1} for the as-prepared unmodified silver nanoparticles. The stability of the catalytic activity of the as-prepared nanozymes was also checked upon their storage at ambient temperature within 7 days at different storage conditions. The results revealed that the peroxidase-like activity of unmodified silver nanoparticles was approximately retained at about 75%, and 63% after 7 days exposing daylight and air oxygen, in order. The shelf-life (storage stability) of the as-prepared nanozymes was also investigated at usual storage conditions (i.e., 4 ℃ under dark), revealed that the nanozymes saved their activity about 96% of their initial activity after 10 days of storage at 4 ℃ under dark conditions.

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Conflict of interest: None.

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