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Research Article

Effect of Daylight and Air Oxygen on Nanozymatic Activity of Unmodified Silver Nanoparticles: Shelf-Stability

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In this work, unmodified silver nanoparticles were synthesized by a simple and cost-efficient method and then characterized by TEM imaging and UV-Vis. spectroscopy. Thereafter, their nanozymatic activity was investigated by catalyzing the oxidation of 3,3',5,5'-tetramethyl-benzidine (TMB) as the standard peroxidase substrate. The results exhibited a specific activity as high as 5.4 µM min^{-1} for the as-prepared unmodified silver nanoparticles. Afterward, the effect of daylight and air oxygen on the peroxidase-like activity of these nanozymes was checked within 7 days. The results revealed that the activity of unmodified silver nanoparticles was approximately retained at about 75%, and 63% after 7 days of exposure to daylight and air oxygen, respectively. The shelf-life of the as-prepared nanozymes was also investigated at 4 ℃ under dark conditions, revealing that these nanozymes retained about 96% of their initial activity after 10 days of storage at 4 ℃ under dark conditions.

Keywords:Unmodified silver nanoparticles; Peroxidase-like nanozymes; Air oxygen; Daylight; Shelf-stability

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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 has caused an enhanced interest in the research on the synthesis and characterization of novel nanomaterials via new methods for achieving 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 also attracted several researchers [12][13]. The new field of catalysis, which was introduced as an alternative to

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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][15][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, to overcome these drawbacks, the enzyme immobilization process has been developed [18][19][20]. Another approach to overcome these difficulties is utilizing the highly stable nanozymes with high enzyme-like activity in enzyme-catalyzed reactions [17]. Among different nanomaterials with enzyme-like activity, noble metal nanoparticles are considered excellent alternatives to enzymes due to their high enzyme-like activity, high stability, and unique green properties [11][14]. Regarding these nanozymes, silver nanoparticles have 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 preserve their optical features and make 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 preserving silver nanoparticles from the significant decrease of optical absorbance during their storage by 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.

2. 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 measurement for buffer preparation. The 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 of sodium citrate (10.0 mM). After that, 89.0 mL of 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 ambient temperature. Finally, yellow-colored silver nanoparticles were stored at 4 ^{o}C under dark conditions for future use.

2.3. Evaluating peroxidase-like activity

To evaluate the peroxidase-like activity of the as-prepared nanozymes, 20 µL of 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 residual activity of nanozymes was calculated by the following formulas [18];

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

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.

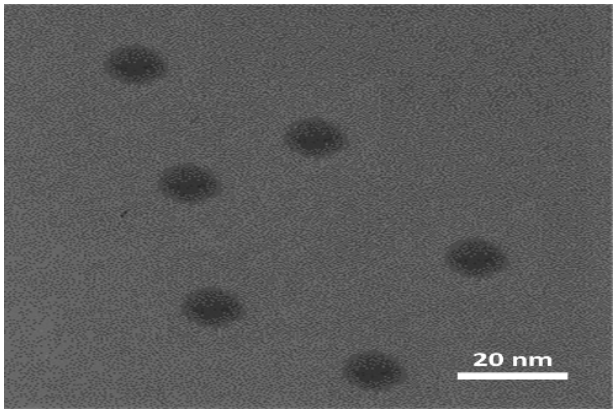

Figure 1. TEM image of as-prepared unmodified silver nanoparticles.

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 at 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][11][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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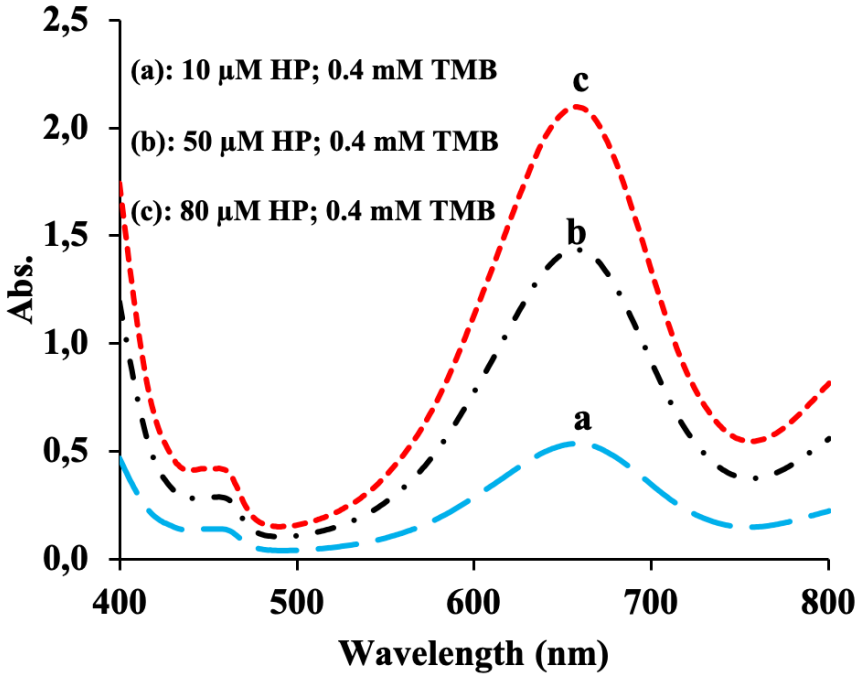

Figure 3. Nanozymatic assay of unmodified silver nanozymes. HP is represented as hydrogen peroxide.

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 the 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 to daylight. The results shown in Figure 3 reveal that the peroxidase-like activity of the as-prepared nanozymes decreased after exposure to daylight and reached about 75% after 7 days of storage. This reduction in activity can be attributed to particle aggregation of nanoparticles by light. The aggregation of the nanoparticles leads to an increase in their size and, consequently, their catalytic performance will reduce. Besides, daylight can catalyze the surface oxidation of these nanoparticles, which causes a reduction in their catalytic activity.

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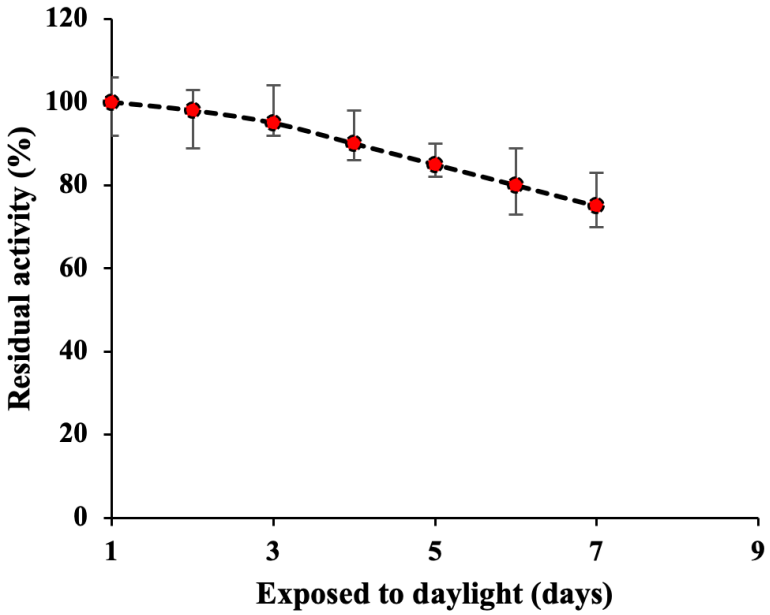

Figure 3. The effect of daylight on peroxidase-like activity of as-prepared nanozymes.

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

In the open air, the 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 was evaluated by their storage in the open air at ambient temperature for 7 days. The activity of the as-prepared nanozymes on the 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 air oxygen. Notably, for exploring more precisely the accuracy of the results, the nanozyme 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 decreased after exposure to air oxygen and reached about 63% after 7 days of storage in the open air.

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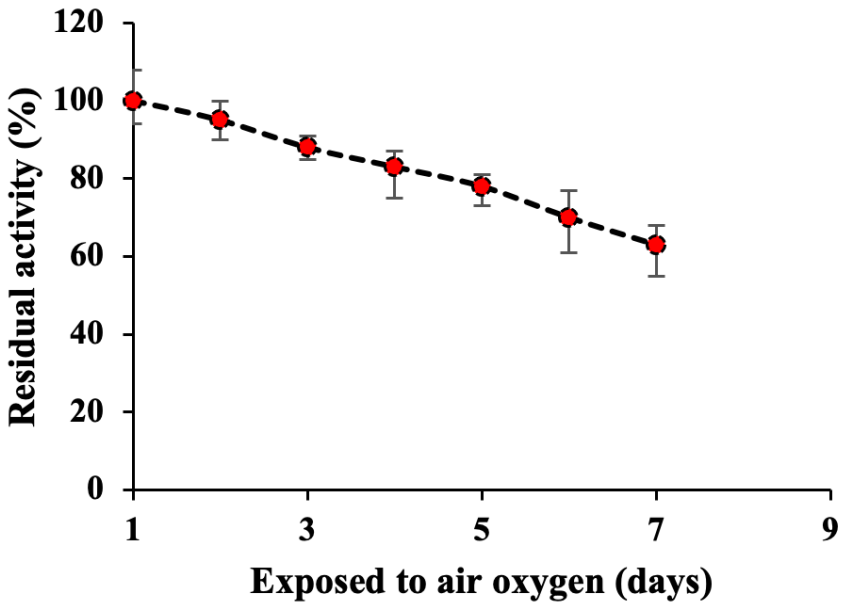

Figure 4. The effect of air oxygen on the peroxidase-like activity of as-prepared nanozymes.

3.5. Storage stability of as-prepared nanozymes

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

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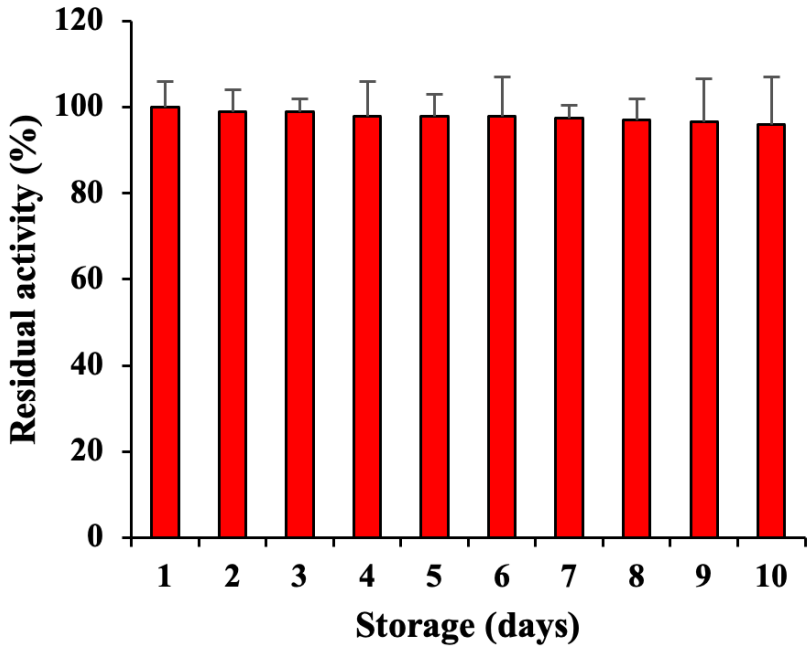

Figure 5. Shelf-life of the as-prepared nanozymes.

4. Conclusions

In this work, unmodified silver nanoparticles were synthesized by a simple and cost-efficient method and then characterized by TEM imaging and UV-Vis. spectroscopy. Thereafter, their nanozymatic activity was investigated by catalyzing the oxidation of 3,3',5,5'-tetramethyl-benzidine (TMB) as the standard peroxidase substrate. The results exhibited a specific activity as high as 5.4 μM min^{-1} for the as-prepared unmodified silver nanoparticles. Afterward, the effect of daylight and air oxygen on the peroxidase-like activity of these nanozymes was checked within 7 days. The results revealed that the activity of unmodified silver nanoparticles was approximately retained at about 75% and 63% after 7 days of exposure to daylight and air oxygen, in order. The shelf-life of the as-prepared nanozymes was also investigated at 4 ℃ under dark conditions, revealing that these nanozymes retained about 96% of their initial activity after 10 days of storage at 4 ℃ under dark conditions.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. a, b, c1. *Hormozi Jangi S. R.; Akhond M. (2020). High thr oughput green reduction of tris (p-nitrophenyl) amine at ambient temperature over homogenous AgNPs as H-transfer catalyst. Journal of Chemical Sciences, 132, 1-8.*
2. a, b, c, dHormozi Jangi, S. R., & Dehghani, Z. (2023). Spe ctrophotometric quantification of hydrogen peroxide utilizing silver nanozyme. Chemical Research and Na nomaterials. https://crn.shiraz.iau.ir/article_701960.ht ml?lang=en.
3. ^Hormozi Jangi, S. R., & Akhond, M. (2021). Ultrasensit ive label-free enantioselective quantification of d-/l-le ucine enantiomers with a novel detection mechanism using an ultra-small high-quantum yield N-doped CD s prepared by a novel highly fast solvent-free method. Sensors and Actuators B: Chemical, 339, 129901.

4. ^Salehzadeh, A., Sadat Shandiz, A., & Naeemi, A. S. (2018). Cytotoxicity effectiveness of biosynthesized silver nanoparticles on breast cancer T47D cell line, using macro algae Laurencia caspica extract. Journal of Ilam University of Medical Sciences, 26(1), 52-61.
5. ^Hormozi Jangi, S. R. (2023). Synthesis and characterization of magnesium-based metal-organic frameworks and investigating the effect of coordination solvent on their biocompatibility. Chemical Research and Nanomaterials, 1(4), 1-9.
6. ^Salata, O. V. (2004). Applications of nanoparticles in biology and medicine. Journal of nanobiotechnology, 2(1), 1-6.
7. ^Sánchez-Moreno, P., De Vicente, J., Nardecchia, S., Marchal, J. A., & Boulaiz, H. (2018). Thermo-sensitive nanomaterials: recent advance in synthesis and biomedical applications. Nanomaterials, 8(11), 935.
8. a, bHormozi Jangi, S. R., Akhond, M., & Absalan, G. (2020). A field-applicable colorimetric assay for notorious explosive triacetone triperoxide through nanozyme-catalyzed irreversible oxidation of 3, 3'-diaminobenzidine. Microchimica Acta, 187, 431.
9. ^Aadil, K. R., Pandey, N., Mussatto, S. I., & Jha, H. (2019). Green synthesis of silver nanoparticles using acacia lignin, their cytotoxicity, catalytic, metal ion sensing capability and antibacterial activity. Journal of Environmental Chemical Engineering, 7(5), 103296.
10. a, bHormozi Jangi, S. R., & Akhond, M. (2020). Synthesis and characterization of a novel metal-organic framework called nanosized electroactive quasi-coral-340 (NEQC-340) and its application for constructing a reusable nanozyme-based sensor for selective and sensitive glutathione quantification. Microchemical Journal, 158, 105328.
11. a, b, cHormozi Jangi, S. R., Akhond, M., & Absalan, G. (2020). A novel selective and sensitive multinanozyme colorimetric method for glutathione detection by using an indamine polymer. Analytica Chimica Acta, 1127, 1-8.
12. a, b, cHormozi Jangi, A. R., Hormozi Jangi, M. R., & Hormozi Jangi, S. R. (2020). Detection mechanism and classification of design principles of peroxidase mimic based colorimetric sensors: A brief overview. Chinese Journal of Chemical Engineering, 28(6), 1492-1503.
13. ^Hormozi Jangi, S. R. (2023). Low-temperature destructive hydrodechlorination of long-chain chlorinated paraffins to diesel and gasoline range hydrocarbons over a novel low-cost reusable ZSM-5@ Al-MCM nanocatalyst: a new approach toward reuse instead of common mineralization. Chemical Papers, 1-15.
14. a, bAkhond, M., Hormozi Jangi, S. R., Barzegar, S., & Absalan, G. (2020). Introducing a nanozyme-based sensor for selective and sensitive detection of mercury (II) using its inhibiting effect on production of an indamine polymer through a stable n-electron irreversible system. Chemical Papers, 74, 1321-1330.
15. ^Hormozi Jangi, S. R., Davoudli, H. K., Delshad, Y., Hormozi Jangi, M. R., & Hormozi Jangi, A. R. H. (2020). A novel and reusable multinanozyme system for sensitive and selective quantification of hydrogen peroxide and highly efficient degradation of organic dye. Surfaces and Interfaces, 21, 100771.
16. ^Ahmadi-Leilakouhi, B., Hormozi Jangi, S. R., & Khorshidi, A. (2023). Introducing a novel photo-induced nanozymatic method for high throughput reusable biodegradation of organic dyes. Chemical Papers, 77(2), 1033-1046.
17. a, bZhou, Y., Liu, B., Yang, R., & Liu, J. (2017). Filling in the gaps between nanozymes and enzymes: challenges and opportunities. Bioconjugate chemistry, 28(12), 2903-2909.
18. a, bHormozi Jangi, S. R., & Akhond, M. (2021). High throughput urease immobilization onto a new metal-organic framework called nanosized electroactive quasi-coral-340 (NEQC-340) for water treatment and safe blood cleaning. Process Biochemistry, 105, 79-90.
19. ^Hormozi Jangi, S. R., & Akhond, M. (2022). Introducing a covalent thiol-based protected immobilized acetylcholinesterase with enhanced enzymatic performances for biosynthesis of esters. Process Biochemistry, 120, 138-155.
20. ^Hormozi Jangi, S. R., Akhond, M., & Dehghani, Z. (2020). High throughput covalent immobilization process for improvement of shelf-life, operational cycles, relative activity in organic media and enzymatic kinetics of urease and its application for urea removal from water samples. Process Biochemistry, 90, 102-112.
21. ^Kavousi, M., & Fatemi, D. (2022). The effect of Spirulina Microalgae extract on Bcl-2 Anti-Apoptotic Gene Expression in Brain Cancer Cell Line. Pars Journal of Medical Sciences, 17(3), 17-23.
22. ^Shanmugaraj, K., & Ilanchelian, M. (2016). Colorimetric determination of sulfide using chitosan-capped silver nanoparticles. Microchimica Acta, 183, 1721-1728.
23. ^Moulaie, S., Mirzaie, A., & Aliasgari, E. (2018). Antibacterial and anticancer activities of silver nanoparticles fabricated by the Artemisia scoparia extract against lung cancer cell line (A549). KAUMS Journal (FEYZ), 22 (5), 487-496.
24. ^Salehzadeh, A., Sadat Shandiz, A., & Naeemi, A. S. (2018). Cytotoxicity effectiveness of biosynthesized silver

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Declarations

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