Micromaterials and Interfaces (2023) Volume 1 Issue 1
doi: 10.54517/mi.v1i1.2170

ORIGINAL RESEARCH ARTICLE

Biochemical characterization of enzyme-like silver nanoparticles toward nanozyme-catalysed oxidation reactions

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ABSTRACT

In this contribution, the biochemical characterization of enzyme-like nanosilvers was performed toward nanozyme-catalyzed oxidation reactions. In this regard, silver nanoparticles were synthesized via a simple chemical reduction method and then characterized by the TEM imaging method. Afterward, their enzyme-like activity was investigated toward catalysis of the oxidation reaction of 3,3',5,5'-tetramethyl-benzidine (TMB) as one of the most popular peroxidase substrates. The results exhibited a specific nanozymatic activity as high as 5400 nM min^{-1} for the as-synthesized nanosilvers toward TMB oxidation. Due to the high enzyme-like activity of the as-prepared nanosilvers, their biochemical properties including pH, thermal, light, and shelf stability were characterized to explore more precisely describing their nanozymatic behavior. The results of thermal and pH stability studies showed that the as-prepared nanosilvers reveal their maximal enzyme-like activity at a wide temperature range of 25 ℃–35 ℃ and a pH range of 3.5–4.5, in order. Regarding the light stability and shelf-life studies, the results exhibited that 75% and 96% of the enzyme-like activity of the as-prepared nanozymes was saved after 7 days exposing visible light and 10 days of storage at 4 ℃ under dark conditions, in order.

Keywords: biochemical characterization; enzyme-like nanosilvers; nanozyme-catalysed reactions; peroxidase mimics

1. 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

ARTICLE INFO
Received: 13 June 2023 | Accepted: 1 August 2023 | Available online: 21 August 2023

CITATION
Hormozi Jangi SR. Biochemical characterization of enzyme-like silver nanoparticles toward nanozyme-catalysed oxidation reactions. *Micromaterials and Interfaces* 2023; 1(1): 2170. doi: 10.54517/mi.v1i1.2170

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]. Another approach for overcoming these difficulties is utilizing the high stable nanozymes with high enzyme-like activity in the enzyme-catalyzed reactions instead of the native enzymes[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, pH, temperature, 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], and *Artemisia scoparia* extract[23] 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 on the biochemical characterization of the catalytic properties of unmodified silver nanoparticles against environmental conditions is limited. In this contribution, silver nanoparticles were synthesized via a simple chemical reduction method and then characterized by the TEM imaging method. Afterward, their enzyme-like activity was investigated toward catalysis of the oxidation reaction of 3,3',5,5'-tetramethyl-benzidine (TMB) as one of the most common peroxidase substrates. Thereafter, due to the high enzyme-like activity of the as-prepared nanosilvers, their biochemical properties including pH, thermal, light, and shelf stability were characterized to explore more precise on their nanozymatic behavior.

2. Experimental section

2.1. Materials and instrumentations

All materials were obtained from Merck Company in their analytical grade. The UV-Visible spectra for nanozymatic activity assay and biochemical characterization of the as-prepared nanosilvers were recorded by An Ultraspec 4000 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 nanosilvers was done by a transmission electron microscope (Zeiss, model EL10C) operated at an accelerating voltage of 80 kV.

2.2. Synthesis of nanosilvers

Silver nanoparticles were synthesized according to the method described by Hormozi Jangi and Akhond[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. Nanozyme activity assay for evaluating peroxidase-like activity of the as-prepared nanosilvers

To quantify the peroxidase-like activity of the as-prepared nanosilvers, 20 µL hydrogen peroxide solution (final concentrations of 50.0 µM or 80.0 µM), and 50 µL of TMB (final concentration in the reaction solution, 0.4 mM), and 80 µL of the as-synthesized nanosilvers were added to 1.0 mL of acetate buffer (0.3 M; pH, 0.4), in order. Afterward, the reaction solution was incubated at ambient temperature for about 10 min for producing the corresponding blue-colored cation radial of TMB (λ_{max} = 658.0 nm) via a 2-electron oxidation pathway[24–26]. After that, the absorbance of the oxidation product (blue-colored) was recorded at the λ_{max} of the oxidation product at 658 nm according to the standard peroxidase mimics activity assay, as reported[2]. The specific activity of nanozymes (µM sec^{-1}) was then calculated using the absorbance coefficient of the oxidation product at 658 nm (ε = 39,000). Notably, the relative and residual activity of the as-prepared nanozymes were calculated by the following formulas[18];

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

$$Relative\ activty = \frac{Activity}{Maximum\ activity} \times 100$$

2.4. pH and thermal stability

The effect of pH on the enzyme-like activity of the as-prepared nanosilvers was estimated by probing their enzyme-like activity over a pH range of pH = 2.0–8.0 using the nanozyme activity assay described in section 2.3. Besides, the thermal stability of the as-mentioned enzyme-like nanosilvers was investigated by calculating their relative activity after incubation at different temperatures over 25.0 ± 1.0 ℃–60.0 ± 1.0 ℃ for 30.0 min.

2.5. Light stability

The effect of visible light on the peroxidase-like activity of as-prepared nanosilvers was evaluated by exposing them to daylight upon storage at ambient temperature for 7 days. The enzyme-like activity of the nanosilvers was then calculated day by day using the nanozyme activity assay described in section 2.3. The activity of the nanosilvers on the first day was considered as control (100%) and then the residual activity of nanozyme upon light exposure was quantified against this control.

2.6. Shelf-life

The shelf-life (shelf stability) of the as-prepared nanosilvers was investigated at usual storage conditions of silver nanoparticles (i.e., 4 ℃ under dark). The activity of the nanosilvers on the first day was considered as control (100%) and then the residual activity of nanozyme upon storage at 4 ℃ was then quantified day-by-day against this control. Notably, details of the nanozyme activity assay were described in section 2.3.

3. Results and discussion

3.1. Characterization of the as-synthesized nanosilvers

In this study, silver nanoparticles were synthesized using a simple chemical reduction method according to the protocol reported by Hormozi Jangi and Akhond[1]. The as-prepared nanosilvers were then 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 reveal a uniform morphology with spherical particles. Concerning the size estimation, a narrow size

distribution over 10.3–12.6 nm with an average size of 11.0 nm was calculated for the as-prepared nanosilvers based on the TEM results.

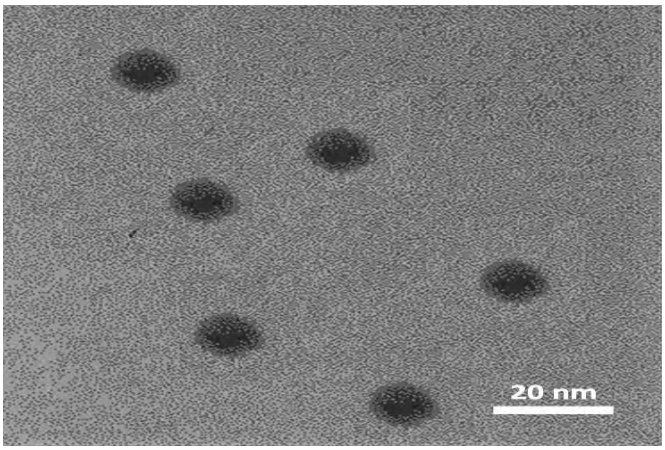

Figure 1. TEM image of the as-prepared nanosilvers.

3.2. Evaluating the peroxidase-like activity of the as-prepared nanosilvers

The peroxidase-like activity of the as-synthesized nanoparticles was investigated using TMB as the peroxidase substrate. Notably, the blue-colored oxidation product of TMB (i.e., TMB-ox) was then utilized as an analytical probe for the quantification of nanozyme activity. The UV-Visible spectra of the oxidation product of 0.4 mM TMB using different concentrations of hydrogen peroxide in the presence of the as-prepared nanosilvers as peroxidase mimics 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 band over 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, nanosilvers produce active hydroxyl radicals by acting on hydrogen peroxide, as previously reported in the literature[2,8,10–12,24,25]. 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 3660 nM min^{-1}, and 5400 nM min^{-1} for 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/nanozyme substrate).

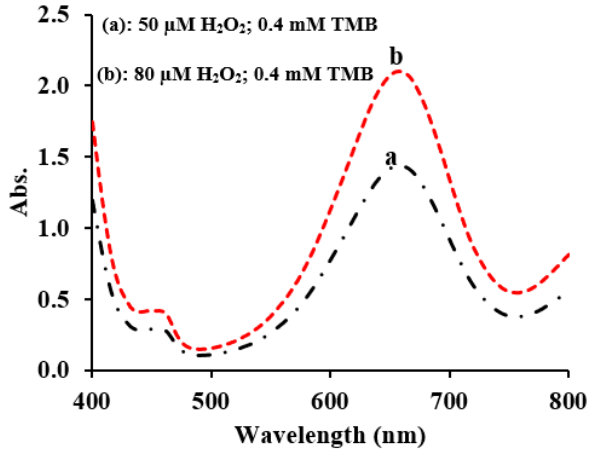

Figure 2. Evaluation of the peroxidase-like activity of the as-synthesized nanosilvers using different concentrations of hydrogen peroxide as the reaction starter.

3.3. pH stability

The pH stability of the as-prepared nanosilvers was evaluated by the determination of their activity as a function of pH variations. To do this the relative activity of the as-prepared nanosilvers was measured over a pH range of 2–8. The results of these tests are shown in **Figure 3**. According to these results, the maximum nanozyme activity of the as-prepared nanosilvers was estimated in the pH range of 3.5–4.5. However, by increasing pH to 4.5, the activity of these nanozymes was decreased and finally reached about 15.7% of its maximal activity in pH = 8.0.

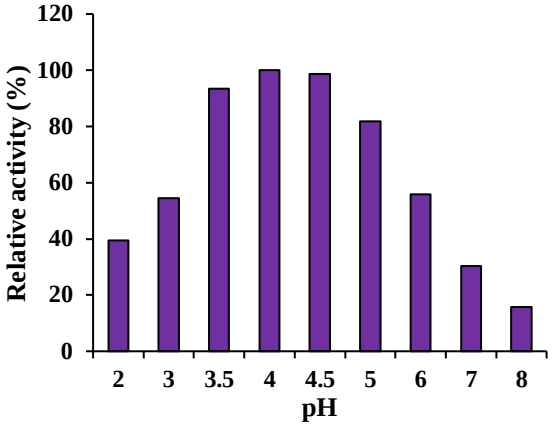

Figure 3. pH stability of the as-synthesized nanosilvers.

3.4. Light stability

The effect of visible light on the peroxidase-like activity of as-prepared nanosilvers 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 visible light. The results shown in **Figure 4** reveal that the peroxidase-like activity of the as-prepared nanozymes was decreased after exposing visible light and reached about 75% after 7 days. 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.

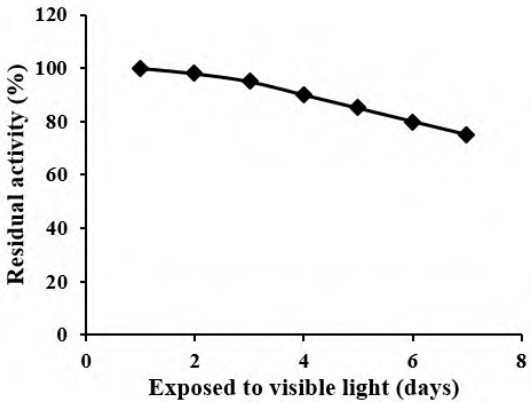

Figure 4. Light stability of the as-synthesized nanosilvers.

3.5. Thermal stability

The thermal stability of the as-prepared nanosilvers was investigated to determine the best performance of peroxidase-like nanosilvers at different temperatures by measuring the relative activity of these nanozymes in a temperature range of 25.0 ± 1.0 ℃–60 ± 1.0 ℃. The plot of nanozyme relative activity as a function of temperature variations was shown in **Figure 5**. According to this figure, the maximum nanozyme activity of the as-prepared nanosilvers was estimated at a wide temperature range of over 25 ℃–30 ℃. It is notable that at 35°C, about 90% of the relative activity of the as-prepared nanozymes has been preserved.

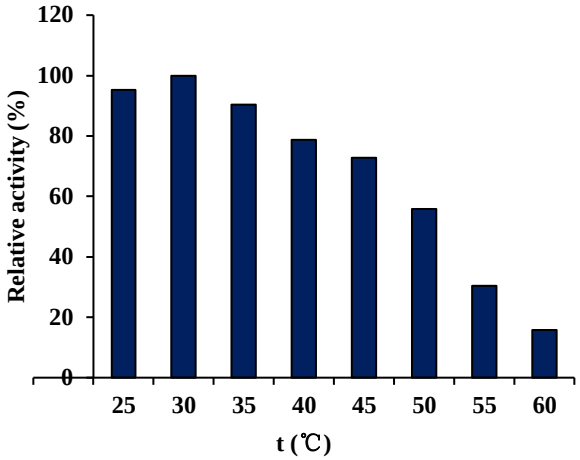

Figure 5. Thermal stability of the as-synthesized nanosilvers.

3.6. Shelf-life

The shelf-life (shelf stability) of the as-prepared nanosilvers was investigated at usual storage conditions of silver nanoparticles (i.e., 4 ℃ under dark). The results are shown in **Figure 6**, 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.

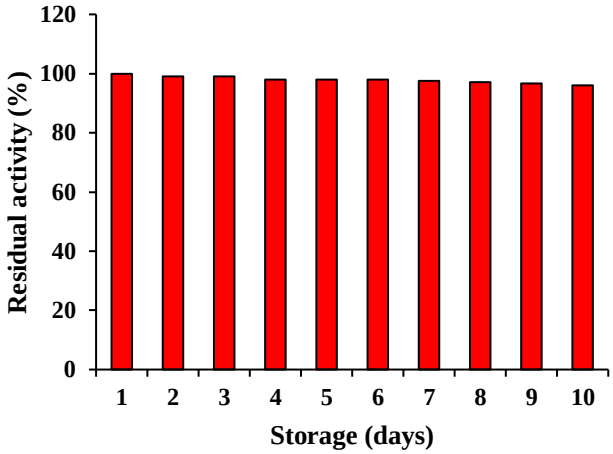

Figure 6. Shelf stability results of the as-synthesized nanosilvers.

4. Conclusions

In this contribution, the biochemical characterization of enzyme-like nanosilvers was performed toward nanozyme-catalyzed oxidation reactions. In this regard, silver nanoparticles were synthesized via a simple chemical reduction method and then characterized by the TEM imaging method. Afterward, their enzyme-like activity was investigated toward catalysis of the oxidation reaction of 3,3',5,5'-tetramethyl-benzidine (TMB) as one of the most common peroxidase substrates. The results exhibited a specific nanozymatic activity as high as 5400 nM min^{-1} for the as-synthesized nanosilvers toward TMB oxidation. Due to the high enzyme-like activity of the as-prepared nanosilvers, their biochemical properties including pH, thermal, light, and shelf stability were characterized for more precisely describing their nanozymatic behavior. The results of thermal and pH stability studies showed that the as-prepared nanosilvers reveal their maximal enzyme-like activity at a wide temperature range of 25 ℃–35 ℃ and a pH range of 3.5–4.5, in order. Regarding the light stability and shelf-life studies, the results exhibited that 75% and 96% of the enzyme-like activity of the as-prepared nanozymes was saved after 7 days exposing visible light and 10 days of storage at 4 ℃ under dark conditions, in order.

Acknowledgments

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

Conflict of interest

The author declares no conflict of interest.

References

1. Hormozi Jangi SR, Akhond M. High throughput green reduction of tris (p-nitrophenyl) amine at ambient temperature over homogenous AgNPs as H-transfer catalyst. *Journal of Chemical Sciences* 2020; 132: 1–8. doi: 10.1007/s12039-020-01819-9
2. Hormozi Jangi SR, Dehghani Z. Spectrophotometric quantification of hydrogen peroxide utilizing silver nanozyme. *Chemical Research and Nanomaterials* 2023; 2(1): 15–23.
3. Hormozi Jangi SR, Akhond M. Ultrasensitive label-free enantioselective quantification of d-/l-leucine enantiomers with a novel detection mechanism using an ultra-small high-quantum yield N-doped CDs prepared by a novel highly fast solvent-free method. *Sensors and Actuators B: Chemical* 2021; 339. doi: 10.1016/j.snb.2021.129901
4. Salehzadeh A, Sadat Shandiz A, Naeemi AS. 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* 2018; 26(1): 52–61.
5. Hormozi Jangi SR. Synthesis and characterization of magnesium-based metal-organic frameworks and investigating the effect of coordination solvent on their biocompatibility. *Chemical Research and Nanomaterials* 2023; 1(4): 1–9.
6. Salata OV. Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology* 2004; 2(1): 1–6. doi: 10.1186/1477-3155-2-3
7. Sánchez-Moreno P, De Vicente J, Nardecchia, S, et al. Thermo-sensitive nanomaterials: Recent advance in synthesis and biomedical applications. *Nanomaterials* 2018; 8(11): 935. doi: 10.3390/nano8110935
8. Hormozi Jangi SR, Akhond M, Absalan G. A field-applicable colorimetric assay for notorious explosive triacetone triperoxide through nanozyme-catalyzed irreversible oxidation of 3, 3′-diaminobenzidine. *Microchimica Acta* 2020; 187: 431. doi: 10.1007/s00604-020-04409-1
9. Aadil KR, Pandey N, Mussatto SI, Jha H. Green synthesis of silver nanoparticles using acacia lignin, their cytotoxicity, catalytic, metal ion sensing capability and antibacterial activity. *Journal of Environmental Chemical Engineering* 2019; 7(5). doi: 10.1016/j.jece.2019.103296
10. Jangi SRH, Akhond M. 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* 2020; 158. doi: 10.1016/j.microc.2020.105328

11. Jangi SRH, Akhond M, Absalan G. A novel selective and sensitive multinanozyme colorimetric method for glutathione detection by using an indamine polymer. *Analytica Chimica Acta* 2020; 1127: 1–8. doi: 10.1016/j.aca.2020.06.012
12. Jangi ARH, Jangi MRH, Jangi SRH. Detection mechanism and classification of design principles of peroxidase mimic based colorimetric sensors: A brief overview. *Chinese Journal of Chemical Engineering* 2020; 28(6): 1492–1503. doi: 10.1016/j.cjche.2020.01.020
13. Hormozi Jangi SR. 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* 2023; 1–15. doi: 10.1007/s11696-023-02834-z
14. Akhond M, Hormozi Jangi SR, Barzegar S, Absalan G. 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* 2020; 74: 1321–1330. doi: 10.1007/s11696-019-00981-w
15. Jangi SRH, Davoudli HK, Delshad Y, et al. A novel and reusable multinanozyme system for sensitive and selective quantification of hydrogen peroxide and highly efficient degradation of organic dye. *Surfaces and Interfaces* 2020; 21. doi: 10.1016/j.surfin.2020.100771
16. Ahmadi-Leilakouhi B, Hormozi Jangi SR, Khorshidi A. Introducing a novel photo-induced nanozymatic method for high throughput reusable biodegradation of organic dyes. *Chemical Papers* 2023; 77(2): 1033–1046. doi: 10.1007/s11696-022-02542-0
17. Zhou Y, Liu B, Yang R, Liu J. Filling in the gaps between nanozymes and enzymes: Challenges and opportunities. *Bioconjugate Chemistry* 2017; 28(12): 2903–2909. doi: 10.1021/acs.bioconjchem.7b00673
18. Jangi SRH, Akhond M. 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* 2021; 105: 79–90. doi: 10.1016/j.procbio.2021.03.027
19. Jangi SRH, Akhond M. Introducing a covalent thiol-based protected immobilized acetylcholinesterase with enhanced enzymatic performances for biosynthesis of esters. *Process Biochemistry* 2022; 120: 138–155. doi: 10.1016/j.procbio.2022.06.004
20. Jangi SRH, Akhond M, Dehghani Z. 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* 2020; 90: 102–112. doi: 10.1016/j.procbio.2019.11.001
21. Kavousi M, Fatemi D. The effect of Spirulina Microalgae extract on Bcl-2 Anti-Apoptotic Gene Expression in Brain Cancer Cell Line. *Pars Journal of Medical Sciences* 2022; 17(3): 17–23. doi: 10.52547/JMJ.17.3.17
22. Shanmugaraj K, Ilanchelian M. Colorimetric determination of sulfide using chitosan-capped silver nanoparticles. *Microchimica Acta* 2016; 183: 1721–1728. doi: 10.1007/s00604-016-1802-y
23. Moulaie S, Mirzaie A, Aliasgari E. Antibacterial and anticancer activities of silver nanoparticles fabricated by the Artemisia scoparia extract against lung cancer cell line (A549). *KAUMS Journal (FEYZ)* 2018; 22(5): 487–496.
24. Jangi SRH. Introducing a high throughput nanozymatic method for eco-friendly nanozyme-mediated degradation of methylene blue in real water media. *Sustainable Chemical Engineering* 2023; 4: 90–99. doi: 10.37256/sce.4220233204
25. Jangi SRH. Determining kinetics parameters of bovine serum albumin-protected gold nanozymes toward different substrates. *Qeios* 2023. doi: 10.32388/07MWBC
26. Hormozi Jangi SR. A comparative study on kinetics performances of BSA-gold nanozymes for nanozyme-mediated oxidation of 3,3’,5,5’-Tetramethylbenzidine and 3,3’-Diaminobenzidine. *Biochemistry and Molecular Biology Journal* 2023; 9: 21. doi: 10.36648/2471-8084-9.03.21