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A new and simple green synthesis of non-toxic, eco-friendly silver nanoparticles by Gum Rosin and antibacterial activity study

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Abstract

Ag nanocolloid was prepared by green synthesis method using gum rosin as a capping and reducing agent and silver nitrate ($AgNO_3$). The prepared Ag nanocolloid was characterized by using different analytical techniques such as UV-Vis, Dynamic light scattering (DLS), Zeta potential, Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and Transmission electron microscopy (TEM). UV–visible spectra of the prepared Ag nanocolloid showed a sharp peak at ~425 nm corresponding to the surface plasmon resonance (SPR) of the silver nanoparticles that were capped with gum rosin molecules. Ag nanoparticles with a diameter of 15-30 nm were observed in TEM and DLS analysis. A negative zeta potential value of −20.54 mV proved the stability of the rosin-stabilized silver nanoparticles. The Ag nanocolloid has shown an antibacterial activity against model organisms, a gram-negative *Escherichia coli* NCIM 2931 in Mueller–Hinton (MH) medium, which is hitherto unattempted up to now. In this study the ratio of Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) (MIC/MBC) was equal to 1, that the green synthesized nanocolloid can be regarded as possessing an antibactericidal performance. The Ag nanocolloid with monodispersed Ag nanoparticles is considered to have potential applications in a variety of fields such as nanosensors, catalysis, electronic devices, and batteries.

Keywords: Ag nanoparticles, Green synthesis, Gum rosin, Antibacterial activity

1. Introduction

In the modern era, rapid and significant improvements and development, and the application capacity of nanotechnology and nanoparticles have attracted remarkable attention and have been extensively studied due to their influential industrial activities and biomedical [1]. Among the nanoparticles in the leading position of the fast-progressing of nanotechnology, silver nanoparticles (AgNPs) have attracted remarkable attention in this field [2,3]. Significantly, biological nanotechnology due to its lower toxicity [4-8] has played a vital role compared to the amendment of chemically synthesized nanoparticles such as microemulsion systems as soft templates [9-11] that need surfactant [12-13].

Kakakhel et al. reported a comprehensive review to describe the biological synthesis of silver nanoparticles that are mostly dependent on plants and microorganisms [3]. Herein, in this study, we have drawn attention toward the new route or direction for the biological synthesis of Ag nanoparticles using a gum.

Gum Rosin, also called colophony or Greek pitch, is a solid and natural form of resin obtained from pines and some other plants, mostly from conifers. It is produced by heating fresh liquid resin to vaporize the volatile liquid terpene components. It is semi-transparent and varies in color from yellow to black. At room temperature rosin is brittle, but it melts at stove-top temperatures. It chiefly consists of different resin acids, especially isomers of Pimaran and Abietan acids. Gum Rosin can be used as an acid using the presence of the carboxyl radical (–COOH).

In this research work, we believe that the rosin as film film-forming material for controlled-release silver nanoparticles and also can be applied as a capping and reducing agent for the formation of silver nanoparticles with a tunable size and morphology. The prepared colloid of silver nanoparticle was characterized with UV-Vis, Dynamic light scattering (DLS), Zeta potential, Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and Transmission electron microscopy (TEM) analysis. As an application the antibacterial activity of Ag colloid was tested against *Escherichia coli* NCIM 2931 in Mueller–Hinton (MH) medium, and Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) were obtained by using a microtitre plate dilution method based on the MTT method.

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2. Experimental

2.1. Material

Silver nitrate ($AgNO_3$) (≥99.0%) and ethanol (99.0%) were purchased from Merck. Chinese gum rosin was received and used as a reducing and stabilizing agent. Deionized water (18 $M\Omega cm^{-1}$) which was produced by the reverse osmosis (RO) water purification process.

2.2. Green synthesis of Ag nanoparticles

In this research work, to prepare Ag nanoparticles based on the green synthesis method, Gum rosin was used as an emulsifier to control of morphology and particle size of obtained silver nanoparticles [3,7].
First, an ethanolic solution of rosin gum (10 ml, 20 mg/L) was prepared and then an aqueous solution of silver nitrate (2 ml, 0.01 M) was added during the 5 min and vigorously stirred for 15 min. After that a clear color change from light yellow to dark brown was observed, confirming the formation of Ag nanoparticles. At the end, 30 ml water was added to the above solution and was characterized with UV-Vis, DLS, Zeta, ICP-OES, and TEM analysis. Fig. 1 shows the prepared Ag nanocolloid with a green and simple route.

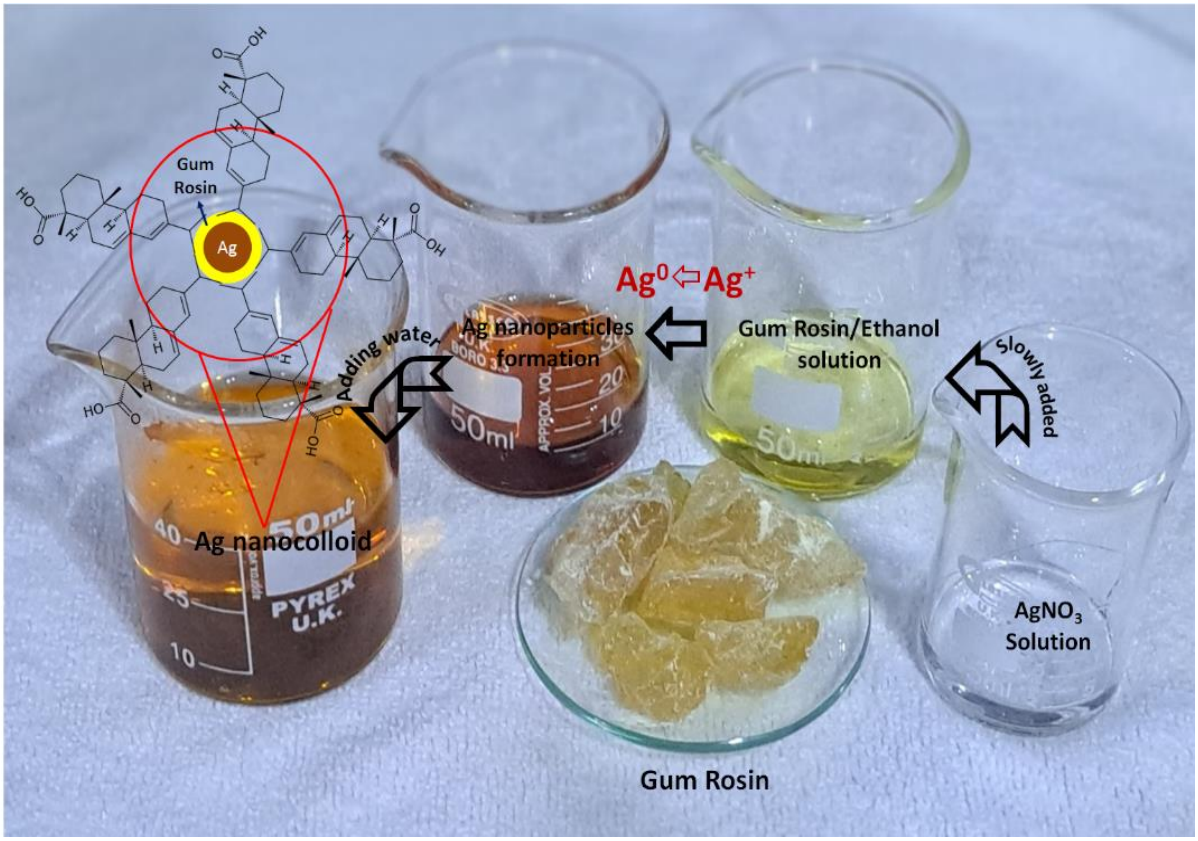

Fig. 1. A schematic image for description of the preparation of the Ag nanocolloid

2.3. Characterization

To confirm the preparation of the Ag nanoparticles, UV-Vis analysis used Analytik Jena Specord 200 Double-Beam Spectrometer Sipper/FlowCell/Peristaltic. DLS was used as a precise measurement technique for characterizing the hydrodynamic size of particles in suspensions and emulsions Malvern Zetasizer Nano ZS. The effective electric charge on the Ag nanoparticles surface and charge quantity was measured with Zeta potential Malvern Zetasizer Nano ZS. TEM was used to directly measure Ag nanoparticle size, grain size, size distribution, and morphology. The electron microscope investigation by coating the sample in a carbon-coated copper grid reflects the size and shape of the AgNPs. ICP-OES (Agilent 5900 ICP-OES) as elemental analysis was used for quantifying determination (total silver content) and the presence of Ag nanoparticles aqueous samples against calibration standards prepared from stock standards over the concentration range of 0.5 – 100 mg/mL.

2.4. Antibacterial study

The antibacterial properties of the produced Ag nanocolloid and ethanolic solution of gum rosin as a control sample were analyzed by the agar well diffusion method [14,15], against a clinical isolate *Eschechia coli* (ATCC 49342) bacterium (from Gram-negative) as representative bacterial strain [16,17]. This procedure was repeated three times. The antibacterial activity assays were analyzed by using pathogens such as Escherichia coli by the method of standard disc diffusion method. In brief, the Mueller–Hinton (MH) medium was used to culture the above-mentioned bacterium [18,19]. Overnight culture of inoculum (100 µl) of culture was spread onto the MH plate. Sterile paper discs of 5 mm diameter (containing different concentrations (µg/ml) of Ag nanoparticles) along with a control sample (without Ag nanoparticles) in the plate. Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) were obtained by using a microtitre plate dilution method based on the MTT method.

3. Result and discussion

3.1. UV-Vis analysis

As can be seen from Fig. 2, this color change is the initial confirmatory test for the formation of Ag nanoparticles and the formation of a deep brown color in the reaction solution is due to Surface Plasmon Resonance (SPR) [20]. A sharp peak at 425 nm revealed the Ag nanoparticles formation [21] (Fig. 2).

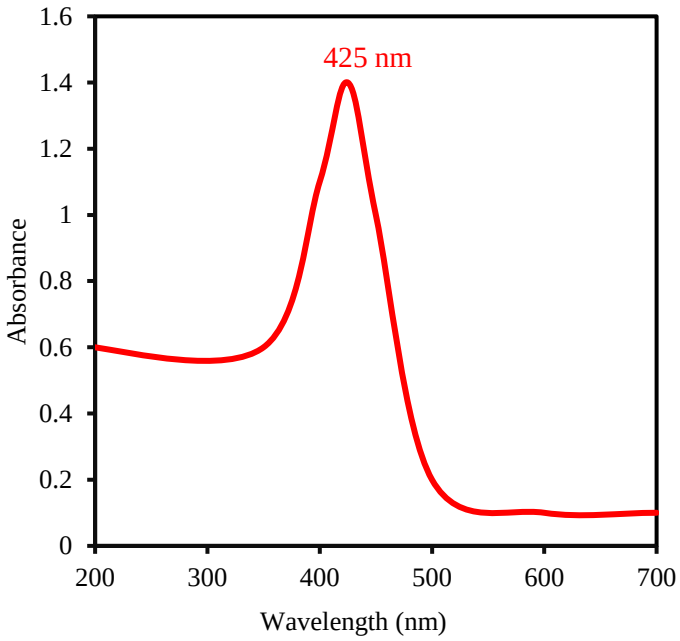

Fig. 2. UV-visible absorption spectrum of Ag nanoparticles stabilized with gum rosin.

3.2. DLS and Zeta potential study

Fig. 3, presents the DLS histogram of Ag nanocolloid and provides accuracy of the particle size and distribution. A common analytical technique associated with nanoparticle sizing is DLS. The DLS analysis indicated an average size (25.66 nm), which was revealed to be stable for 14 days and also confirmed the small size of the particles without agglomeration. Zeta-potential is a useful instrument to determine the stability of nanoparticles and the surface charge based on the electrical potential developed at the solid–liquid interface in response to the relative nanoparticle movement and the solvent [22].

To study the zeta potential, the prepared silver nanocolloid was sonicated for 2 min. The zeta potential of prepared gum rosin-modified silver nanoparticle is -20.54 mV and depends upon the electrical potential and the surface charge and the surface potential of charged particle increases with an increase in zeta potential [23]. So, it is very important to know them. The negative charge is due to gum rosin which chiefly consists of different resin acids, especially isomers of Pimaran and Abietan acids, and the presence of the carboxyl radical (–COOH). Hence, the negative charge confirmed that the silver nanoparticles capped and stabilized with gum rosin molecules and gum rosin acts as a surfactant.

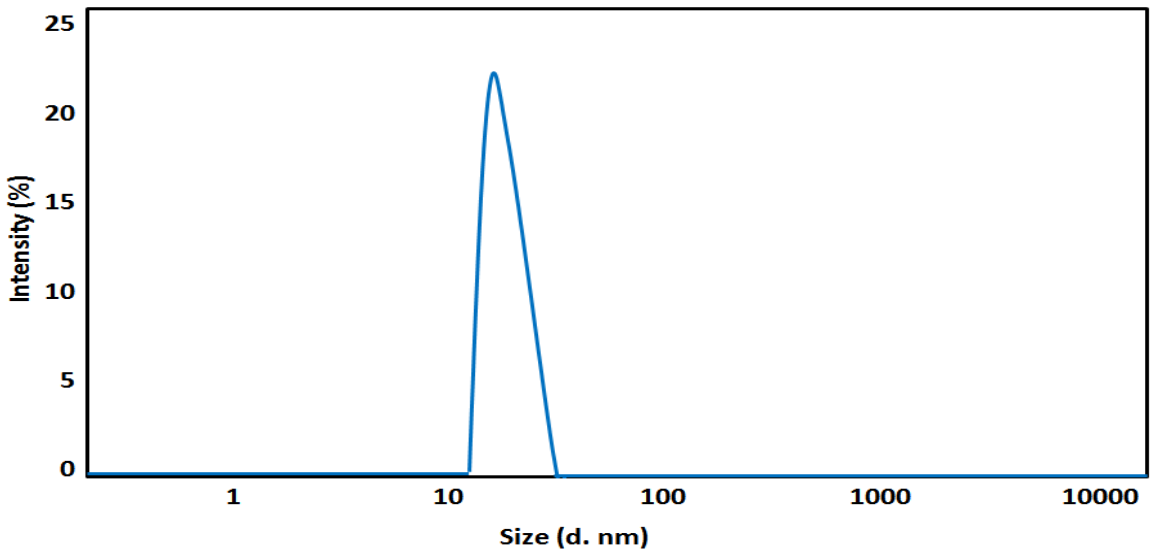

Fig. 3. DLS image of Ag nanoparticles stabilized with gum rosin and the size of the nanoparticles is 25.66 nm.

3.3. ICP-OES

ICP-OES is an analytical technique used to determine how much of certain elements are in a sample. The ICP-OES principle uses the fact that atoms and ions can absorb energy to move electrons from the ground state to an excited state. Before the analysis of the real sample, a Quality control (QC) test was done to confirm the accuracy and validity of ICP-OES report. The result of ICP-OES shows that the concentration of Ag in colloid is 125 mg/L.

3.4. TEM

TEM image (Fig. 4) of the colloidal solution of Ag nanoparticles show that they are monomorphic, predominantly contained particles with a spherical outline and from size around 15–30 nm. This data also supported and statistically correlated with DLS analysis.

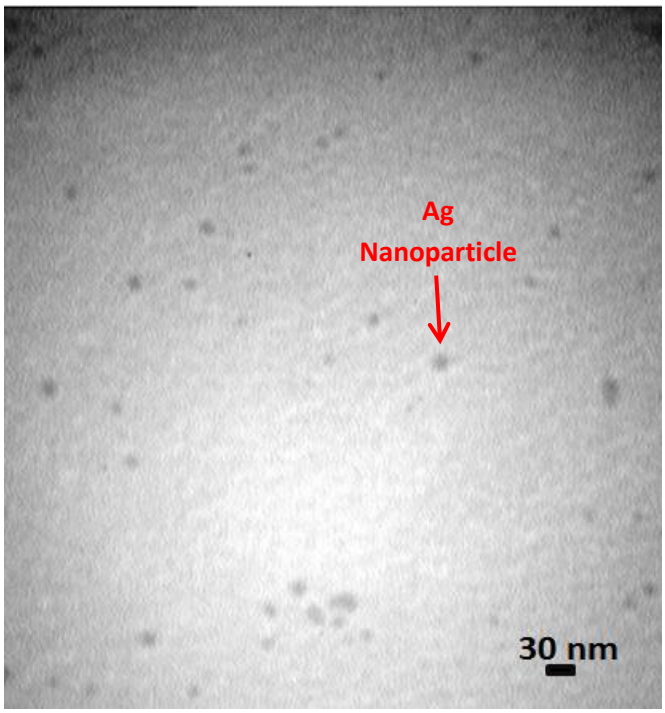

Fig. 4. TEM image of Ag nanoparticles stabilized with gum rosin

3.5. Antibacterial activity study

As an application in biomedicine, Ag nanoparticles have been employed on various surfaces of devices and instruments such as prostheses and catheters to prevent bacterial colonization to reduce microbial infection in

burn wounds [24,25]. Hence in the present study, the antibacterial activity of green-synthesized Ag nanocolloid against gram-negative model organism, Escherichia coli NCIM 2931 was evaluated and summarized in Tab. 1 with different concentrations of Ag nanocolloids.

To study whether the as-synthesized Ag nanocolloid possesses antibacterial performance and, since the resulting Ag nanocolloid in this work is very stable and forms a fine colloid in MH broth (we did not observe settlement of the particles for about 24 h), an antibacterial investigation was performed by modification of the CLSI document M2-A9 26:1.

A zone of inhibition of (18 mm diameter) growth of Escherichia coli around the disc for 60 µg/ml of the Ag nanocolloid in contrast to diluted -ethanolic gum rosin as control sample without Ag nanoparticles (7 mm diameter), only for qualitative and comparative purposes.

The silver nanocolloids have been reported to possess an antibacterial activity. However, as per our knowledge, there are no reports of the antibacterial activity of Ag nanocolloid prepared in solution of gum rosin. Hence it is the first parallel work to report an antibacterial activity of a synthesized Ag nanocolloid. So, in the context of the previous discussion, it is apparent to discuss the antibacterial activity of Ag nanocolloid that was prepared by the new method based on the gum rosin as a capping and reducing agent. In this research work, the MIC and MBC values of Ag nanocolloid were studied, which respectively are the lowest concentrations at which a tested compound, inhibits growth or kills more than 3 log (99.9%) of the bacteria. Based on the obtained results, both the MIC and MBC value of Ag nanocolloid was 60 µg/ml. At this concentration, there was a 3-log reduction in the number of viable cells. So, with an increase in the concentration of the nanoparticles, there was a decrease in the CFU/ml of the Escherichia coli. Cornstarch/hyaluronic acid/ ethanolic extract of propolis (CS/HA/EEP) as Film dressings prepared by solvent-casting and the CS/HA/0.5%EEP film dressing exhibited higher antibacterial activity against Staphylococcus aureus (2.08 ± 0.14 mm), Escherichia coli (2.64 ± 0.18 mm), and Staphylococcus epidermidis (1.02 ± 0.15 mm) in comparison with the CS, CS/HA, and CS/HA/0.25%EEP films [19].

The ratio of MIC/MBC is important in deciding the fate of nanoparticles as a static (to inhibit) and tidal (to kill) agent in this work for Ag nanocolloid, the ratio of MIC/MBC was 1.

For a ratio of, >1, the nanoparticles as bacteriostatic, while a ratio of 1 or less, is categorized it as bactericidal. Since in this study, the ratio of MIC/MBC was equal to 1, the green synthesized nanocolloid can be regarded as possessing an antibactericidal performance [22,28, 29].

The bactericidal mechanism of Ag nanocolloid against bacteria is not very well-known to researchers. But, a possible contribution to the bactericidal activity of silver nanoparticles in colloids is the release of silver ions from the nanometric particles. Another bactericidal effect is generally recognized that silver nanoparticles may penetrate inside the cell with attachment to the bacterium and cause damage by interacting with phosphorus and sulfur-containing compounds such as DNA and protein attach to the cell wall, thus disturbing cell-wall permeability [30]. The antibacterial study of Ag nanocolloid was found to be exciting and hence further antifungal activity study is in progress in our laboratory.

Table 1. Antibacterial activity of the silver nanocolloid based on disk diffusion method

Samples	Zone of inhibition (mm in diameter)					
	10	15	25	35	45	60
Ag nanocolloid	-	3±0.15	5±0.55	10±0.60	12±0.02	18±0.71
Control	-	-	-	3±0.27	5±0.31	7±0.12

4. Conclusion

The green synthesis method is eco-friendly, of low cost and capable of producing Ag nanoparticles at room temperature. Here, for the first time, gum rosin act as both reducing and stabilizing agents. The Ag nanocolloid were characterized by UV–Vis, DLS-Zeta potential, ICP-OES and TEM analysis. The surface plasmon resonance of green-synthesized Ag nanocolloid confirmed with the UV–Vis spectral. Gum rosin as natural resin is a biomolecule was responsible for reducing and capping of Ag nanoparticles, which were confirmed by DLS-Zeta measurements. TEM studies revealed spherical and uniform-shaped silver nanoparticles with size in the range 20–50 nm. The biosynthesized Ag nanoparticles were found to have a pronounced antibacterial activity against *E. coli* based on the MIC and MBC test. In this current research work, proteins and flavonoids in the gum rosin play an important role in the formation of silver nanoparticles.

Conflicts of Interest

The author declares no conflict of interest.

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References

[1] M.A. Kakakhel, W. Sajjad, F. Wu, N. Bibi, K. Shah, Z. Yali, W. Wang, W. Green synthesis of silver nanoparticles and their shortcomings, animal blood a potential source for silver nanoparticles: A review. J. Hazard. Mater. Advances, 1(2021), 100005. https://doi.org/10.1016/j.hazadv.2021.100005

[2] Y. Geng, H. Khodadadi, A. Karimipour, M. Reza Safaei, T.K. Nguyen, A comprehensive presentation on nanoparticles electrical conductivity of nanofluids: Statistical study concerned effects of temperature, nanoparticles type and solid volume concentration. Physica A, 542 (2020) 123432. https://doi.org/10.1016/j.physa.2019.123432.

[3] N.R. Barveen, T.-J. Wang, Y.-H. Chang, Photochemical decoration of silver nanoparticles on silver vanadate nanorods as an efficient SERS probe for ultrasensitive detection of chloramphenicol residue in real samples. Chemosphere, 275 (2021) 130115. https://doi.org/10.1016/j.chemosphere.2021.130115

[4] C. Vanlalveni, S. Lallianrawna, A. Biswas, M. Selvaraj, B. Changmai and S. L. Rokhum, Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: a review of recent literature, RSC Adv., 11 (2021) 2804-2837. https://doi.org/10.1039/D0RA09941D

[5] Hemlata, P.R. Meena, A.P. Singh, K.K. Tejavath, Biosynthesis of silver nanoparticles using *cucumis prophetarum* aqueous leaf extract and their antibacterial and antiproliferative activity against cancer cell lines, ACS Omega 5 (2020) 5520–5528. https://doi.org/10.1021/acsomega.0c00155

[6] A. Giri, B. Jena, B. Biswal, A.K. Pradhan, M. Arakha, S. Acharya, L. Acharya, Green synthesis and characterization of silver nanoparticles using *Eugenia roxburghii* DC. extract and activity against biofilm-producing bacteria. Sci. Rep., 12 (2022) 8383. https://doi.org/10.1038/s41598-022-12484-y

[7] R. Gengan, K. Anand, A. Phulukdaree, A. Chuturgoon, A549 lung cell line activity of biosynthesized silver nanoparticles using Albizia adianthifolia leaf, Colloids Surf., B 105 (2013) 87–91, https://doi.org/10.1016/j.colsurfb.2012.12.044

[8] A. Salabat, F. Mirhoseini, M. Mahdieh, H. Saydi, A novel nanotube-shaped polypyrrole-Pd composite prepared using reverse microemulsion polymerization and its evaluation as an antibacterial agent, New J. Chem. 39 (5) (2015) 4109–4114. https://doi.org/10.1039/c5nj00175g

[9] A. Salabat, F. Mirhoseini, R. Valirasti, Engineering poly(methyl methacrylate)/Fe_2O_3 hollow nanospheres composite prepared in microemulsion system as a recyclable adsorbent for removal of benzothiophene, Ind. Eng. Chem. Research 58 (2019) 17850-1785. https://doi.org/10.1021/acs.iecr.9b04322

[10] A. Salabat, F. Mirhoseini, K. Abdoli, A microemulsion route to fabrication of mono and bimetallic Cu/Zn/γ-Al_2O_3 nanocatalysts for hydrogenation reaction. Scientia Iranica, 25(2018) 1364-1370. https://doi.org/10.24200/sci.2018.5023.1048

[11] F. Mirhoseini, A. Salabat, Removal of methyl tert -butyl ether as a water pollutant by photodegradation over a new type of poly(methyl methacrylate)/TiO_2 nanocomposite. Polymer Composites, *39*(4) (2018) 1248–1254. https://doi.org/10.1002/pc.24059

[12] F. Alimohamadi, A. Salabat, Effect of surfactant type on the particle dispersion in Ag/polystyrene nanocomposite prepared by microemulsion method, Colloid Nanosci. J. 1 (2023) 112-118. https://doi.org/10.52547/CNJ.1.2.112

[13] F. Mirhoseini, A. Salabat, Ionic liquid based microemulsion method for fabrication of poly(methyl methacrylate)–TiO_2 nanocomposite as highly efficient visible light photocatalyst, RSC Adv. 5 (2015) 12536–12545. https://doi.org/10.1039/c4ra14612c

[14] F. Mirhoseini, Alireza Salabat, Iinvestigation of operational parameters on the photocatalytic activity of a new type of poly(methyl methacrylate)/ionic liquid-tio_2 nanocomposite, Iranian J. Chem. Chem. Eng., 38 (2019) 101-114. https://doi.org/10.30492/IJCCE.2019.37613

[15] F. Kamali, K. Faghihi, F. Mirhoseini, High antibacterial activity of new eco-friendly and biocompatible polyurethane nanocomposites based on Fe_3O_4/Ag and starch moieties. Polym. Eng. Sci., 62(5) (2022) 1444-1462.https://doi.org/10.1002/pen.25934

[16] A. Salabat, F. Mirhoseini, Applications of a new type of poly(methyl methacrylate)/TiO_2 nanocomposite as an antibacterial agent and a reducing photocatalyst. Photochem. Photobiol. Sci., 14(9) (2015) 1637–1643. https://doi.org/10.1039/c5pp00065c

[17] F. Mirhoseini, A. Salabat, Antibactrial activity based poly(methyl methacrylate) supported TiO_2 photocatalyst film nanocomposite, Tech. J. Eng. Appl. Sci. 5 (2015)115-118.

[18] A. Eskandarinia, A. Kefayat, M. Rafienia, M. Agheb, S. Navid, K. Ebrahimpour, Cornstarch-based wound dressing incorporated with hyaluronic acid and propolis: In vitro and in vivo studies. Carbohydr. Polym. 216, (2019) 25–35. https://doi.org/10.1016/j.carbpol.2019.03.091
[19] A. Salabat, F. Mirhoseini, F.H. Nouri, Microemulsion strategy for preparation of TiO_2–Ag/poly(methyl methacrylate) nanocomposite and its photodegradation application. J. Iranian Chem. Soc. 20 (2022) 599–608. https://doi.org/10.1007/s13738-022-02693-7.
[20] A. Salabat, F. Mirhoseini, M. Arjomandzadegan, E. Jiryaei, A novel methodology for fabrication of Ag-polypyrrole core-shell nanosphere using microemulsion system and evaluation of its antibacterial application, New J. Chem. 41 (21) (2017) 12892–12900. https://doi.org/10.1039/c7nj00678k
[21] B.S. Mirhoseini, A. Salabat, A novel surfactant-free microemulsion system for the synthesis of poly(methyl methacrylate)/Ag nanocomposite, J. Mol. Liq. 342 (2021) 117555. https://doi.org/10.1016/j.molliq.2021.117555
[22] A. Salabat, F. Mirhoseini, A novel and simple microemulsion method for synthesis of biocompatible functionalized gold nanoparticles, J. Mol. Liq. 268 (2018) 849–853. https://doi.org/10.1016/j.molliq.2018.07.112
[23] A. Salabat, F. Mirhoseini, Photo-induced hydrophilicity study of poly(methyl methacrylate)/TiO_2 nanocomposite prepared in ionic liquid based microemulsion system. Current Appl. Polym. Sci., 2(2), (2018) 112–120. https://doi.org/10.2174/2452271602666180803141554
[24] A. Salabat, F. Mirhoseini, Polymer-based nanocomposites fabricated by microemulsion method, Polym. Compos. 43 (2022) 1282–94. https://doi.org/10.1002/pc.26504
[25] F. Mirhoseini, A. Salabat, Photocatalytic filter, United States patent 10828629.
[26] F. Mirhoseini, A. Salabat, Polymer nanocomposite based composition and method for controlling water hardness, United States patent 11136247
[27] M. Saravanan, A.K. Vemu, S.K. Barik, Rapid biosynthesis of silver nanoparticles from bacillus megaterium (ncim 2326) and their antibacterial activity on multi drug resistant clinical pathogens, Colloids Surf. B Biointerfaces 88 (2011) 325–331. https://doi.org/10.1016/j.colsurfb.2011.07.009
[28] M. Saravanan, A. Nanda, Extracellular synthesis of silver bionanoparticles from Aspergillus clavatus and its antimicrobial activity against MRSA and MRSE. Colloids Surf. B Biointerfaces 77 (2010) 214–218. https://doi.org/10.1016/j.colsurfb.2010.01.026
[29] M Behzadi, S Jarollahi, M Ahsani Irvani, D Ghanbari, Green synthesis and antibacterial activity of silver nanoparticles using dracocephalum moldavica leaves extract, JNS 12 (4) (2022) 1059-1066.
[30] A. Salabat, F. Mirhoseini, Z. Masoumi, M. Mahdie, Preparation and characterization of polystyrene-silver nanocomposite using microemulsion method and its antibacterial activity, JNS 4 (2014) 377-382.