Synthesis And Antibacterial Activity Of Zno And Fe Incorporated Zno: A Comparative Study

Mohammad Faid Khan[1], Pushpendra Kumar[2]*, Amit K. Chaturvedi[3]*

[1,2,3]Department of Chemistry, J S University, Shikohabad, UP, 283135– India. [2]Email: pushpendra.kmr1@gmail.com, [3]Email: achaturvedi794@gmail.com

***Corresponding Author:** Pushpendra Kumar, Amit K. Chaturvedi
*Department of Chemistry, J S University, Shikohabad, UP, 283135– India. Email: pushpendra.kmr1@gmail.com, Email: achaturvedi794@gmail.com

Abstract:
Oxide nanocomposites have attracted much attention due to their potential application in environmental remediation. Among various semiconductor system, Zinc Oxide (ZnO) has been widely used for competent disinfection and antimicrobial activity. Reports reveal that by incorporation of foreign particles may modify the host material by inducing favourable properties. In the present study, ZnO and Fe incorporated ZnO were synthesized by sol-gel co-precipitation method. Synthesized material was characterized by X-Ray diffraction (XRD) for crystalline structure, UV spectroscopy for optical behaviour, scanning electron microscopy and energy dispersive spectroscopy for morphological and elemental investigation. Various characterizations show the formation of hexagonal wurtzite phase of ZnO and Fe incorporated ZnO. To investigate the antibacterial properties of ZnO and Fe doped ZnO, two gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa) & two gram positive bacteria (Staphylococcus aureus Bacillus subtilis) were chosen and agar well diffusion method was adopted to investigate antibacterial activity. Compare to plane ZnO, Fe doped ZnO showed significant antibacterial activity.

Keywords: Fe doped ZnO, antibacterial activity

Introduction

The resistance of bacteria against antibiotics is one of the major problems[1]. In order to overcome this prolem, research has been turned up towards nanotechnology as nanoparticles can enter the body of bacteria and rupture the cell wall of bacteria[2]. Metal or their oxides are emerging as an effective material due to their antibacterial activity. But the poor efficiency of simple metal oxide is still a matter of concern. To enhance the activity, now a days, combitorial approach is being used in which doping, nanocomposites, mixtures of multiple phase are used as a tailoring tool. However, the selection of material plays a vital role. ZnO and ZnO based nanocompsites have been widely used as a powerful antibacterial agent for various strain[3]. The nanoparticles of zinc oxide in the range 30-40 nm in diameter can enter the bacterial cell without damage of cell wall/ cell membrane but encourages the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which induce the biochemical alteration in cell leading to death of bacterium[4]. Silver (Ag), gold (Au), Iron (Fe) have been reported for antibacterial properties and exploited for treating various ailments[5-6]. To increase the effectiveness of ZnO, it can be combined with any other favourable material or can be doped by suitable metal having comparable ionic radii. When any foreign particle enter the crystal lattice or get combined with host material, it changes the electron transport system (increase the electron mobility) and also generate intraband gap states.

In this study, Zinc ferrite was synthesized as the ionic radii of zinc ion and iron ion have comparable values and can get combined with ZnO easily. The microstructural properties were determined by various characterization techniques. To study the antibacterial behaviour of zinc ferrite, it was applied against four different bacterial strains along with zantamycin as an standard. The results are encouraging which shows that nanocomposites can play a vital role against bacterial strains.

Experimental

To synthesize the ZnO, 10 g Zinc acetate dihydrate was dissolved in 250 ml water and stirred for 30 minutes. Then NaOH solution was added dropwise to maintain the pH of the solution around 10. White precipitate was formed and the whole solution was stirred for 4 hours at 60 °C. The precipitate was filtered out and washed with water for many times. Finally precipitate was kept in muffle furnace at 500 °C for 1 hour. The precipitate was allowed to cool slowly overnight. To introduce Iron, Iron nitrate was used as a dopant and similar procedure was followed.

The prepared material was subjected to X-ray analysis for microcrystalline characterisation and UV spectroscopy for optical properties. The surface morphology was investigated by scanning electron microscope while elemental composition was determined by energy dispersive spectroscopy.

To investigated antibacterial activity agar well diffusion methods was chosen and applied on two different strains of gram-negative bacteria as well as two different strains of gram-positive bacteria. Selected bacterial strains were procured from IMTECH, Chandigarh, India. Escherichia coli and Pseudomonas aeruginosa both are gram-negative, as well as Staphylococcus aureus and Bacillus subtilis two gram-positive bacteria, were used to test the antibacterial activity. Different test concentration was applied to investigate the effectiveness of material towards antibacterial activity.

Results and Discussion

1. Microstructural properties

In the present study, ZnO and $ZnFe_2O_3$ was synthesized by sol-gel coprecipitation method. Zinc acetate was used as a precursor for Zn^{2+} ions while iron nitrate was used for the source of iron. NaOH was utilized to enhance the pH and to improve the formation of ZnO. On addition of an OH^- ion may led the formation of tiny hydroxide oligomers[7]. On heating at higher temperature metal ions separate from the hydroxide and form the initial metal oxide crystallites embedded in gel. High temperature during annealing also promotes a proper crystallization by providing enough thermal energy for crystallization[8]. Figure 1 shows a X-ray diffraction pattern of ZnO and $ZnFe_2O_3$. The presence of multiple peaks confirms the polycrystalline nature of the material. Major peaks at 2θ values 31.7, 34.5, 36.1, 39.3, 43.1, 47.5, 56.5, 62.8, 67.9° confirms the presence of Hexagonal wurtzite phase of ZnO (PDF – 01-071-6424). In case of Fe incorporated ZnO, the additional peak at 2θ, 35.23° shows the magnetite phase of Fe_2O_3. No other additional peak was observed which confirms the proper incorporation of iron in crystal lattice of ZnO. The ionic radii of Zn^{2+} and Fe^{2+} are comparable (76 pm) and that's why proper incorporation was expected. Peak at 2θ, 35.23° may be arisen due to higher concentration of Fe which do not get incorporated in ZnO crystal lattice. Utilizing the XRD data and Scherrer's calculations (Eq 1) average grain size was calculated[9].

$$\tau = k\,\lambda / B \cos\theta \qquad (1)$$

Where, t is grain size, B is the full width at half maxima and λ is the wavelength of X-ray used (1.548 Å).

The average crystalline size of ZnO was found to be 48 nm while that of Fe doped ZnO was 33 nm. From the XRD pattern, it can be seen that on Fe incorporation, the peaks get slightly broadened. Also peak intensity of Fe incorporated ZnO was also reduced. In both the samples, sharp peaks shows good crystallinity of samples. However, broadness of XRD peaks in Fe doped ZnO depicts the slight distortion in lattice which is expected [10]. When any foreign particle enters the crystal lattice, the host lattice parameters contracts/expand to accommodate the dopant which may lead to strain in the lattice and may cause broadening of peaks..

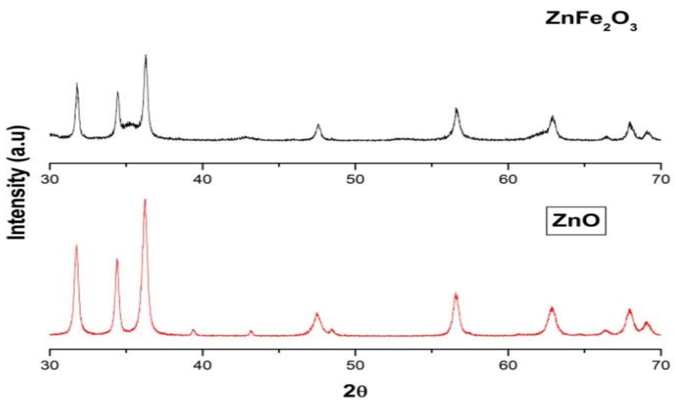

Figure 1: X-Ray diffraction pattern of ZnO and Fe incorporated ZnO

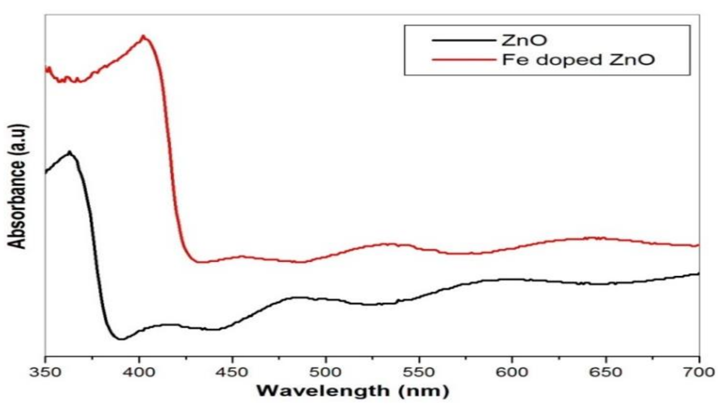

Figure 2: Optical absorption of ZnO and Fe incorporated ZnO

Figure 2 depicts the plot of optical absorption against the wavelength. ZnO showed strong optical absorption in UV region while in case of Fe doped ZnO, the absorption shifted towards higher wavelength (towards visible region). Whenever any foreign particle is introduced in crystal lattice, depending upon the nature of dopant particle it generates intraband gap states which results lowering in band gap of the material[11]. From the absorption plot, it can be seen that on doping of Fe in ZnO, the absorption edge shifted from 380 nm to 440 nm i.e red shift and also optical absorption increased.

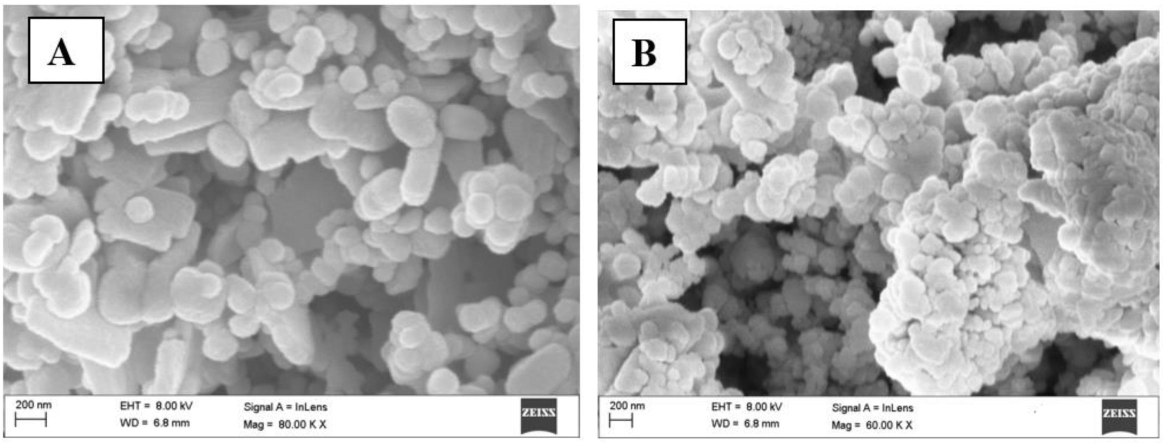

Figure 3: Surface morphology of ZnO (A) and Fe doped ZnO (B)

Figure 3 shows the surface morphology of ZnO and Fe incorporated ZnO. In both the samples, clear grain boundaries can be seen. The particles of pure ZnO are comparable bigger than Fe doped ZnO which are in agreement with the size calculated from X-ray diffraction. The samples are free from any major deformity or defect. Uniform distribution of particles can be seen. On doping of Fe, the size of ZnO particles reduced which shows increase in surface area which may offer better contact area resulting in better antibacterial activity. So doping is good option for modification of crystalline properties.

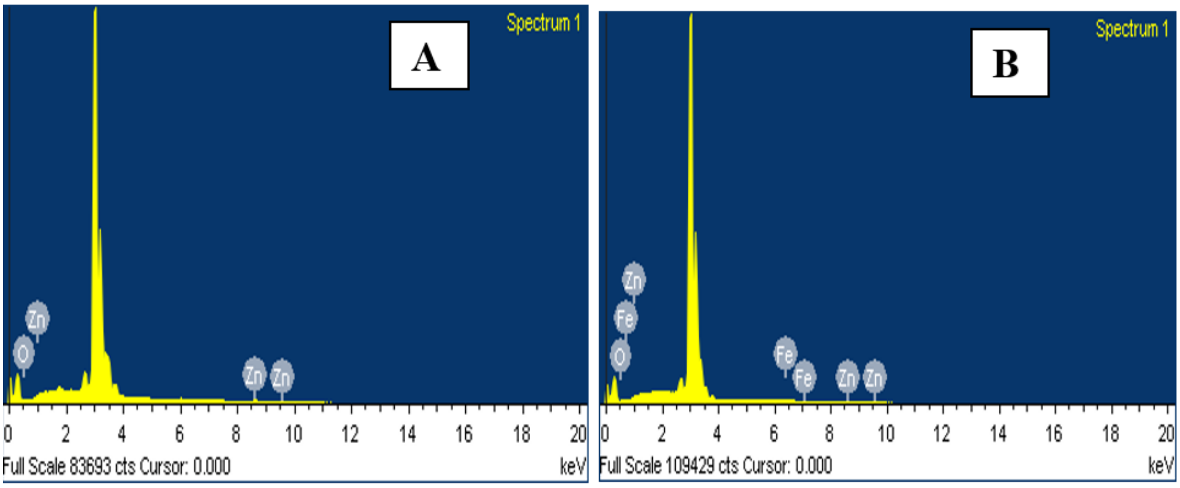

Figure 4: Energy dispersive spectra (elemental composition) of ZnO (A) and Fe doped ZnO

For elemental composition, an energy dispersive spectrum (EDS) was recorded. EDS of ZnO shows pure form of ZnO in which no element other than Zn and O was observed while in case of Fe incorporated ZnO, alongwith Zn and O, Fe is also present. This shows the incorporation of Fe in ZnO. As per the EDS spectra the concentration of Fe was found to be 15.94 At%.

2. Comparative antibacterial activity of ZnO and Fe doped ZnO

ZnO possesses remarkable antimicrobial activity and is also biocompatible, making it an attractive candidate for application as an antibacterial agent. ZnO nanoparticles, in particular, have been shown to have effective bactericidal properties against both Gram-positive and Gram-negative bacteria. However, the specific antibacterial mechanism of ZnO is not fully understood, which limits the applicability of ZnO as an antibacterial material to only a subset of its potential uses. Investigations into the antibacterial mechanism of ZnO materials would advance medical research and open up new avenues of medical investigation[12,13]. To increase the activity of oxide materials, various dopant have been used. In current study, we introduced Fe in ZnO crystal and its effect on antibacterial properties was investigated.

Surface area and concentration determine antibacterial activity, while crystalline structure and particle shape have little effect. The greater the concentration and surface area, greater the antibacterial activity[15].

The antibacterial activity of ZnO and Fe-ZnO was Escherichia coli and Pseudomonas aeruginosa both are gram-negative, as well as Staphylococcus aureus, Bacillus subtilis two gram-positive bacteria, were used to test the antibacterial activity. To study the antibacterial activity, the starting dose of 5mg/ml of material was taken. The holes of 6 mm were made using the sterile borer, 100 µl of test solutions were added in the plate with different concentration of ZnO nanoparticles and Fe-Znonano particles. After incubation for 24 hours at 37^0C, the inhibition zone was evaluated and measured. Experiments were conducted in triplicate for measurements of zone of inhibition, followed by mean calculation and standard deviation analysis [16].

Table:1 Antibacterial analysis of ZnO nanoparticles and Fe-ZnO on gram-positive and gram-negative bacterial strains. Results are showing mean value of triplicate with standard deviation

Test Materials (Nanoparticles)	**Test concentration**	**Test Microorganisms (Zone of inhibition in mm with standard deviation in well diffusion method)**			
		E. coli	***Pseudomonas aeuroginosa***	***Staphyllococcus aureus***	***Bacillus subtillis***
Zno-NPs (5mg/ml)	100 µl	14.2 ± 0.6	22.4 ± 0.6	27.4 ± 0.5	16.1 ± 0.5
Zno-NPs (5mg/ml	200 µl	17.2 ± 0.8	22.9 ± 0.7	28.2 ± 0.3	18.9 ± 0.8
Fe-ZnO NPs (5mg/ml)	100 µl	18.2 ± 0.3	23.7 ± 0.6	27.9 ± 0.8	17.2 ± 0.4
Fe-ZnO NPs (5mg/ml)	200 µl	22.7 ± 0.6	25.3 ± 0.9	28.4 ± 0.6	18.7 ± 0.6
Fe-ZnO NPs (5mg/ml)	300 µl	25.6 ± 0.8	26.8 ± 0.6	29.2 ± 0.3	19.9 ± 0.6
Fe-ZnO NPs (5mg/ml)	400 µl	27.1 ± 0.6	28.1 ± 0.4	29.8 ± 0.6	20.5 ± 0.5
Fe-ZnO NPs (5mg/ml)	500 µl	27.3 ± 0.5	28.2 ± 0.6	30.0 ± 0.2	20.7 ± 0.2
Gentamicin (5 mg/ml)	100 µl	20.1 ± 0.7	15.2 ± 0.4	24.6 ± 0.3	22.3 ± 0.6
DMSO	100 µl	0	0	0	0

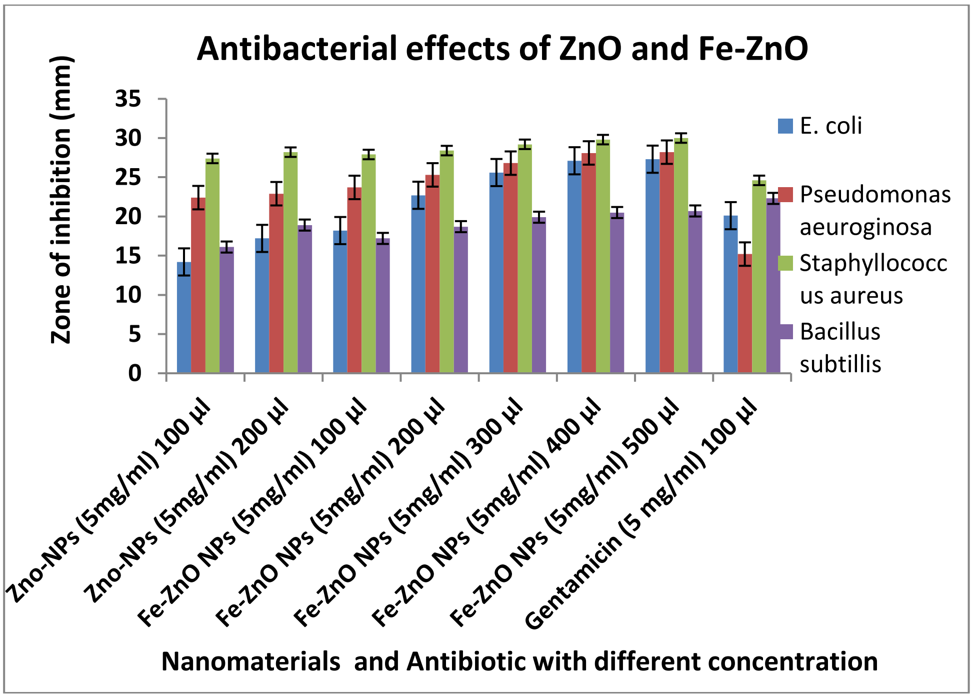

Figure:5 Antibacterial effets of Zno and FE-ZnO nanoparticles on gram-positive and gram-negative bacteria. (Standard error bar of mean value is shown with p=0.05)

From the figure 5, it can be seen that as the test concentration increased zone of inhibition increased. Compared to plane ZnO, Fe incorporated ZnO showed better response. As per XRD analysis, the crystalline size decreased on Fe dopingwhich in turn increase the surface area resulting better antibacterial activity.

Conclusion:

ZnO and Fe incorporated ZnO were successfully synthesized by sol-gel coprecipitation method. Exhaustive evolution of hexagonal wurtzite phase alongwith Fe_2O_3 was observed. To study antibacterial activity of synthesized material agar well diffusion method was employed. Compared to plane ZnO, Fe incorporated ZnO showed better antibacterial activity. However, more study on material modification is required to increase the efficiency and to understand the specific mechanism of antibacterial activity.

Reference:

1. Peterson E and Kaur P (2018), Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. Front. Microbiol. 9:2928. doi: 10.3389/fmicb.2018.02928
2. Yael N. Slavin, Jason Asnis, Urs O. Häfeli and Horacio Bach (2017), Metal nanoparticles: understanding the mechanisms behind antibacterial activity, J Nanobiotechnology, 15: 65. doi: 10.1186/s12951-017-0308-z
3. Roberta C. de Souza, Leticia U. Haberbeck, Humberto G. Riella, Deise H. B. Ribeiro and Bruno A. M. Carciofi (2019), Antibacterial activity of zinc oxide nanoparticles synthesized by solochemical process, Brazilian Journal of Chemical Engineering, 36: 885 – 893, dx.doi.org/10.1590/0104-6632.20190362s20180027.
4. Amna Sirelkhatim, Shahrom Mahmud, Azman Seeni, Noor Haida Mohamad Kaus, Ling Chuo Ann, Siti Khadijah Mohd Bakhori, Habsah Hasan, and Dasmawati Mohamad (2015), Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism, Nanomicro Lett., 7, 219–242, doi: 10.1007/s40820-015-0040-x
5. Ankush Chauhan, Ritesh Verma, Swati Kumari, Anand Sharma, Pooja Shandilya, Xiangkai Li, Khalid Mujasam Batoo, Ahamad Imran, Saurabh Kulshrestha, Rajesh Kumar (2020), Photocatalytic dye degradation and antimicrobial activities of Pure and Ag-doped ZnO using Cannabis sativa leaf extract, Sci Rep, 10, 7881, https://doi.org/10.1038/s41598-020-64419-0
6. Trilok K. Pathak, R.E. Kroon, Valentin Craciun, Marcela Popa,M.C. Chifiriuc and H.C. Swart (2019), Influence of Ag, Au and Pd noble metals doping on structural, optical and antimicrobial properties of zinc oxide and titanium dioxide nanomaterials, Heliyon. 5(3): e01333, doi: 10.1016/j.heliyon.2019.e01333.
7. Naofumi Uekawa, Ryo Yamashita,Yong Jun Wu and Kazuyuki Kakegawa (2004), Effect of alkali metal hydroxide on formation processes of zinc oxide crystallites from aqueous solutions containing $Zn(OH)_4^{2-}$ ions, Phys. Chem. Chem. Phys., 6, 442-446, doi: https://doi.org/10.1039/B310306D
8. Lan Wang, Guilin Liu, Xi Xi, Guofeng Yang, Lifa Hu, Bingjie Zhu, Yifeng He, Yushen Liu, Hongqiang Qian, Shude Zhang and Huachao Zai (2022), Annealing Engineering in the Growth of Perovskite Grains, Crystals, 12(7), 894; https://doi.org/10.3390/cryst12070894
9. P. Bindu and Sabu Thomas (2015), Estimation of lattice strain in ZnO nanoparticles: X-ray peak profile analysis, Journal of Theoretical and Applied Physics, 8, 123–134, doi: https://doi.org/10.1007/s40094-014-0141-9
10. Bidyarani Maibam, Saptaka Baruah & Sanjeev Kumar (2020), Photoluminescence and intrinsic ferromagnetism of Fe doped zinc oxide, SN Appl. Sci., 2, 1712, https://doi.org/10.1007/s42452-020-03519-y
11. Maria Sygletou, Stefania Benedetti, Alessandro di Bona, Maurizio Canepa,and Francesco Bisio (2022), Doping-Dependent Optical Response of a Hybrid Transparent Conductive Oxide/Plasmonic Medium, J Phys Chem C Nanomater Interfaces., 126 (4): 1881–1889. doi: 10.1021/acs.jpcc.1c07567
12. Kezhen Qi, Bei Cheng, Jiaguo Yu, Wingkei Ho (2017), Review on the improvement of the photocatalytic and antibacterial activities of ZnO, Journal of Alloys and Compounds, 727, 792-820, doi: https://doi.org/10.1016/j.jallcom.2017.08.142.
13. Thana ShugaAldeen, HamzaElsayed Ahmed Mohamed, Malik Maaza (2022), ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity, Journal of Physics and Chemistry of Solids, 160, 110313, https://doi.org/10.1016/j.jpcs.2021.110313.
14. Yamamoto O., M. Hotta, J. Sawai, T. Sasamoto& H. Kojima (1998), Influence of powder characteristic of ZnO on antibacterial activity effect of specific surface area. J. Ceram. Soc. Japan, 106, 1007–1011
15. Rafael Álvarez-Chimal, Víctor I. García-Pérez, Marco Antonio Álvarez-Pérez, Rosario Tavera-Hernández, Lorena Reyes-Carmona, Miryam Martínez-Hernández, Jesús Ángel Arenas-Alatorre (2022), Influence of the particle size on the antibacterial activity of green synthesized zinc oxide nanoparticles using Dysphania ambrosioides extract, supported by molecular docking analysis, Arabian Journal of Chemistry, 15, 103804, https://doi.org/10.1016/j.arabjc.2022.103804
16. Renata Dobrucka and Jolanta Długaszewska (2016), Biosynthesis and antibacterial activity of ZnO nanoparticles using Trifolium pratense flower extract, Saudi Journal of Biological Sciences, 23, 517-523, doi: https://doi.org/10.1016/j.sjbs.2015.05.016