MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

Formulation and Evaluation of ZnO-loaded levofloxacin nanoparticles to fight *Salmonella typhi*

Sumangla Kadian
Research Scholar
Department of Chemistry,
Baba Mastnath University, Asthal Bohar, Rohtak, Haryana

Dr. Heena Dahiya
Assistant Professor
Department of Chemistry,
Baba Mastnath University, Asthal Bohar, Rohtak, Haryana

Dr. Sunil Kumar Jangra
Assistant Professor
Department of Chemistry,
AIJHM College, Rohtak, Haryana

Abstract

Nanomaterials containing inorganic metallic ions are commonly employed to treat various illnesses, including cancer, autoimmune disorders, and bacterial and fungal colonization. In this study, Zn-loaded levofloxacin nanoparticles (ZLFNs) are used as an antibacterial agent. The adsorption-produced nanocarriers were examined using FTIR, SEM, TEM, and HPLC. Simulated body fluid was used in in vitro release experiments at 37 °C. Data were evaluated using a variety of kinetic models, which revealed a slow dissolution for 12 to 24 hours. Research on antimicrobial agents revealed enhanced efficaciousness against *Salmonella typhi*. It was experimentally found that ZLFNs possessed a particle size of 140.1 nm with a zeta potential value of -19.8 mV and had a good encapsulation efficiency of 87-91%.

Keywords: Levofloxacin; Toxicity; Antimicrobial; *Salmonella typhi*

Introduction

Nanotechnology is still fresh in biomedical and pharmaceutical technology. Nanomaterials, especially imaging agents and drug carriers, are promising medical applications due to their large surface area, greater tissue penetration, drug degradation protection, and drug delivery [1-3]. Recently, cutting-edge drug delivery systems have used carbon-based nanomaterials such as nanotubes, graphene, and fullerenes, as well as metal oxide nanoparticles [4-5].

Levofloxacin is a broad-spectrum fluoroquinolone antibiotic. Healthcare professionals use it to treat skin, urinary tract, respiratory, and other bacterial infections. Blocking the bacterial DNA gyrase enzyme prevents DNA supercoiling and disrupts DNA replication and repair. This

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

kills bacteria [6]. One of levofloxacin's key benefits is its high bioavailability, which allows for uncomplicated oral administration. Its long half-life allows once-daily dosage in many cases. To conclude, levofloxacin treats bacteria well [7].

The antibacterial properties of Zinc oxide (ZnO) have garnered attention in recent years. ZnO is used in industry, biology, and healthcare [8-10]. It is increasingly involved in antibacterial agents. ZnO's antibacterial properties stem from its ability to create ROS when wet. ROS like superoxide ions and hydroxyl radicals harm bacterial cells' lipids, proteins, and DNA through oxidative stress. Oxidative damage kills bacteria and affects vital biological processes [11]. ZnO kills Gram-positive and Gram-negative bacteria, according to research. Its antimicrobial activity has been tested in medical device coatings, wound healing, and water treatment [12]. Because they have shown promising results against antibiotic-resistant bacteria, ZnO nanoparticles may be useful in combating antibiotic resistance [13-15].

Typhoid fever, caused by *Salmonella typhi*, is a major public health issue in places with poor water and sanitation [16]. Typhoid fever is spread by direct contact with ill persons and contaminated food and water. Salmonella typhi bacteria enter the bloodstream through the intestinal mucosa and cause systemic disease. The bacteria can then spread to the spleen, liver, and bone marrow, where they can damage tissue and cause inflammation [17-19].

Without timely treatment, severe cases can cause intestinal perforation, septicemia, and death. Typhoid fever is treated with third-generation cephalosporins or fluoroquinolones to kill the bacteria [20-22]. However, drug-resistant *Salmonella typhi* strains make therapeutic efforts difficult, emphasizing the need for monitoring and antimicrobial stewardship.

Clean water, sanitation, and hygiene are needed to prevent typhoid fever in addition to immunization. Visitors to endemic locations should have *Salmonella typhi* immunizations [23]. These include the injectable Vi polysaccharide and oral live-attenuated Ty21a vaccines. Immunization, sanitation, and antibiotic use are employed in prevention and control [24].

This study uses zinc oxide (ZnO)-coated levofloxacin (ZLFNs) nanoparticles as antibacterial agents. These nanoparticles were characterized using TEM, zeta sizer, and FTIR [25-27]. ZnO-loaded levofloxacin (ZLF) nanoparticles were tested for drug loading and encapsulation using HPLC. This evaluation was used to collect and display the pharmaceutical release profile.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

MATERIALS AND METHODS

Materials: We bought ZnO (purity > 99%) from MP Biomedicals, LLC in France. The levofloxacin (purity > 99%) source was Sigma Aldrich India. The materials used in this research project were of the analytical or reagent variety. We obtained *Salmonella typhi* from MTTC Chandigarh.

Preparation of the ZnO-loaded levofloxacin

First, 120 mg of zinc oxide (ZnO) was accurately weighed and mixed with 150 mg of levofloxacin in 4 milliliters of sterile water. The mixture was then subjected to ultrasonic oscillations for a period of 30 minutes to ensure thorough blending and interaction between the ZnO and levofloxacin. After the sonication process, the resulting mixture was carefully washed several times using deionized water to remove any impurities or unreacted materials.

Following the washing process, the mixture was subjected to freeze-drying (lyophilization) overnight to completely remove the water content and obtain a dry powdered product. This process yielded the ZnO-levofloxacin antibacterial system. The final product was then analyzed using particle size analysis (PSA) by employing dynamic light scattering (DLS) to evaluate the size distribution and characteristics of the particles in the synthesized antibacterial system. [28].

Characterization of synthesized ZnO-loaded levofloxacin.

Particle size, entrapment efficiency, zeta potential, morphology, FTIR, and in-vitro drug release were all evaluated for ZLFNs [29]. The average size of the nanoparticles, along with their size distribution, was measured using dynamic light scattering (DLS) to assess the characteristics of the optimized nanoformulations. The polydispersity index (PDI) was used to evaluate the distribution of particle sizes, with a lower PDI indicating a more uniform size distribution. The measurements were performed using a Zetasizer Nano ZS, a device that utilizes dynamic light scattering technology to measure particle sizes in suspension. The temperature during the analysis was maintained at 25°C to ensure consistent and accurate results. From this analysis, the average particle size of the zinc oxide-levofloxacin nanoparticles (ZLFNs) was determined, providing crucial data for understanding the formulation's behavior in various applications, such as drug delivery or antibacterial systems. [30].

The drug loading and drug entrapment efficiency of the formulated nanoparticles were determined by quantifying the amount of drug present in the collected supernatant after the formulation process. This was accomplished using high-performance liquid chromatography

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

(HPLC), a sensitive and precise analytical technique commonly employed to separate, identify, and quantify components in a mixture. For the analysis, after the formulation process, the nanoparticles were subjected to centrifugation to separate the nanoparticles from the supernatant. The collected supernatant, which contains any unencapsulated or unbound drug, was analyzed using HPLC to determine the concentration of the drug that was not entrapped within the nanoparticles.

Drug loading refers to the percentage of drug successfully incorporated into the nanoparticles relative to the total amount of nanoparticles. Drug entrapment efficiency represents the percentage of the initial amount of drug that was successfully encapsulated within the nanoparticle matrix. Both of these parameters are critical in evaluating the effectiveness and efficiency of the nanoparticle formulation for drug delivery purposes. [31].

The morphology of the optimized batch was studied using TEM and SEM. The smallest structures in matter can be seen using the analytical method known as TEM. By magnifying nanoscale structures up to 50 million times, TEM may reveal astonishing detail at the atomic scale, in contrast to optical microscopes, which rely on light in the visible range. SEM is frequently used to look into the chemistry and microstructure of many materials [32].

To characterize and confirm the interactions between zinc oxide (ZnO), levofloxacin, and the synthesized ZnO-levofloxacin nanoparticles (ZLFNs), Fourier transform infrared (FT-IR) spectroscopy was employed. The powdered samples of ZnO, levofloxacin, and ZLFNs were individually analyzed by preparing them as potassium bromide (KBr) pellets. This involved thoroughly mixing the samples with KBr powder, which acts as a transparent matrix for infrared light, and pressing the mixture into a pellet form. The FT-IR spectroscopy analysis was conducted over a wavenumber range of 4500–500 cm^{-1}, which allows for the detection of various functional groups and bonding interactions within the samples. This wide spectral range ensures the identification of key absorption peaks corresponding to specific chemical bonds and molecular structures present in the materials.

By comparing the FT-IR spectra of the pure ZnO, pure levofloxacin, and the ZnO-levofloxacin nanoparticles, any shifts in absorption peaks or the appearance of new peaks could be observed. These spectral changes help to confirm the formation of the ZnO-levofloxacin complex and indicate any potential chemical interactions or bonding that occurred between the drug and the ZnO nanoparticles during the synthesis process. [33].

Using HPLC, the amount of ZnO released from the NPs under in vitro conditions was ascertained. The release kinetics were investigated using the dialysis sac technique. In

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

summary, 10 ml of 0.1 M (pH 7.4) was washed down to dissolve 10 mg of ZnO and ZnO-loaded levofloxacin nanoparticles. These were then put into a dialysis sac and submerged in 250 ml of phosphate buffer saline. A thermostat was used to continually agitate the release medium at 80 rpm. At regular intervals of 0, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42, and 48 hours, 1 ml of sample was taken. Using standard curves at 254 nm, the samples were examined to investigate the release of levofloxacin nanoparticles loaded with ZnO [34].

Antibacterial activity

The Agar well diffusion method will be used to measure the antibacterial activity against *Salmonella typhi*. A volume of the microbial inoculum was applied to the whole surface of the agar plate to inoculate it. Subsequently, a sterile cork borer or tip was used to punch an aseptic hole measuring 6 to 8 mm in diameter. A volume of 20 to 100 µL of the necessary concentration of the antimicrobial agent or extract solution was then added to the well. After that, agar plates were incubated in the appropriate environment for the test microorganism. The studied microbial strain's growth is inhibited by the antimicrobial agent as it diffuses throughout the agar media [35].

RESULTS AND DISCUSSION

Particle size and Zeta potential

The particle size and zeta potential of the ZnO-levofloxacin nanoparticles (ZLFNs) were measured to assess the physical properties and stability of the synthesized nanoformulations. Particle size measurements were conducted using dynamic light scattering (DLS), which provides an accurate estimation of the size distribution of nanoparticles in suspension. The analysis revealed that the average size of the produced nanoparticles was 140.1 nm, as shown in Figure 1. This nanoscale size is crucial for ensuring efficient cellular uptake, making the ZLFNs suitable for drug delivery applications. In addition to particle size, the zeta potential of the ZLFNs was measured to evaluate the surface charge of the nanoparticles. The zeta potential is an indicator of the electrostatic stability of colloidal suspensions; higher magnitudes (positive or negative) generally suggest greater stability due to electrostatic repulsion between particles, which reduces the tendency for aggregation.The zeta potential of the ZLFNs was determined to be -19.8 mV, as illustrated in Figure 2. This negative value indicates that the surface of the nanoparticles carries a net negative charge, which contributes to the stability of the formulation by preventing particle agglomeration. A zeta potential value in this range suggests that the nanoparticles are in a relatively stable state, with sufficient repulsive forces to maintain dispersion in the medium. Stability is crucial for the nanoparticles to retain their desired properties during storage and when administered in biological environments.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

In vitro release profile of ZLFNs

The incorporation of levofloxacin and zinc oxide (ZnO) into the nanoparticle formulation effectively protects the drug from rapid metabolism and degradation. This protection is achieved through the rate-controlled release of the drug from the polymeric matrix of the nanoparticles. Unlike the rapid release often seen in unmodified drug formulations, the nanoparticle matrix allows for a more controlled, sustained release, prolonging the drug's presence in the system and enhancing its therapeutic effectiveness.

According to in-vitro drug release studies, pure ZnO exhibited a fast release profile, with 84.44% of the ZnO being released within just three hours. This rapid release would typically limit the drug's effectiveness due to premature metabolism and degradation. However, in the case of the ZnO-levofloxacin nanoparticles (ZLFNs), a much slower, continuous release was observed. After three hours, only 38.49% of the ZnO was released from the ZLFNs, and after 24 hours, the release had gradually increased to 76.22%. This extended release pattern is advantageous for drug delivery, as it maintains a therapeutic concentration of the drug over a longer period of time, reducing the need for frequent dosing.

The sustained release of ZnO from the ZLFNs can be attributed to several factors. One key factor is the hydrophobic (nonpolar) nature of magnesium oxide, which slows down the diffusion of the drug through the nanoparticle matrix, resulting in a more gradual release over time. Additionally, levofloxacin plays a crucial role in controlling the release kinetics. By forming a dense matrix around the ZnO particles, levofloxacin acts like a "cage" with thick walls, encapsulating the ZnO. This dense structure ensures that the ZnO is released slowly and continuously, preventing rapid diffusion and providing sustained therapeutic action.

This controlled release profile of ZLFNs demonstrates the effectiveness of nanoparticle-based drug delivery systems in overcoming the challenges of rapid drug metabolism and degradation, allowing for more efficient and prolonged drug activity in the body.

Percentage encapsulation efficiency

The type of encapsulating material is another crucial factor affecting encapsulation efficiency. Different polymers and materials possess distinct properties that influence their interaction with the drug molecules. For instance, polymers with hydrophilic characteristics may facilitate better encapsulation of polar drugs, while hydrophobic materials may be more suitable for nonpolar drugs.Moreover, the media used during the encapsulation process can impact the stability and solubility of both the drug and the encapsulating materials. The choice of solvent and conditions, such as temperature and pH, can affect how well the drug is integrated into the nanoparticle structure.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

For the ZLFNs, the encapsulation efficiency was found to range from 87% to 91%. This high efficiency indicates that a significant proportion of the levofloxacin was successfully incorporated into the nanoparticle matrix, maximizing the therapeutic potential of the formulation. The efficiency values reflect the effectiveness of the encapsulation strategy, ensuring that a substantial amount of the drug is retained within the nanoparticles during storage and delivery, thus enhancing the overall efficacy of the therapeutic system. High encapsulation efficiency is desirable, as it minimizes waste and improves the economic viability of drug formulations for clinical applications. (Fig 3).

Morphological characterization of ZLFNs by TEM, and SEM

Drug release, solubility, and dissolution rates are influenced by the size, shape, and dimensions of nanoparticles [36]. Migration of nanoparticles, based on size, shape, and dimension, to different body parts [37-38]. The spherical, segregated ZLFNs were discovered to have a particle size range of 25–46 nm (Fig. 4). Size variation was noted in the nanoparticles that were estimated by PSA and TEM. This is because the TEM operates on the principles of the particle dimension in the isolated atmosphere, while the PSA principle operates on the particle's ionic mobility [39]. The spherical forms of the nanoparticles were verified by SEM image analysis (Fig 5).

FTIR Analysis of Drug Samples

FTIR spectroscopy was employed to validate the loading of ZnO in levofloxacin nanoparticles (ZLFNs) and to infer ZnO-loaded levofloxacin ZLFNs interaction investigations [30]. The absorption bands at 3328 cm -1 for -OH, which represent intermolecular H-bonding, and the stretching bonds at 2843 cm -1 and 2191 cm -1, which represent the terminal -CH3 groups, are visible in the FTIR spectrum of ZnO (Fig. 6). In the FTIR spectra, levofloxacin and magnesium oxide exhibit distinctive peaks [40]. Bands remained unchanged despite a drop in peak intensity. signifying there is no chemical bond between ZnO and levofloxacin.

Antibacterial Activity

The antibacterial properties of the ZnO-levofloxacin nanoparticles (ZLFNs) were evaluated using *Salmonella typhi*, a bacterium known to cause typhoid fever and a significant public health concern worldwide. As illustrated in Figure 7, the efficacy of ZLFNs in inhibiting the growth of S. typhi was tested and compared to that of pure zinc oxide (ZnO) and levofloxacin used as controls.

The results indicated that ZLFNs exhibited superior antibacterial potency compared to both ZnO and levofloxacin alone. This enhanced antibacterial activity can be attributed to several factors. Firstly, the nanoparticles possess a significantly larger surface area-to-volume ratio

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

compared to their bulk counterparts. This increased surface area allows for more active sites available for interaction with bacterial cells, facilitating more effective contact and interaction with the pathogen.

Additionally, the combination of ZnO and levofloxacin within the nanoparticle matrix may create a synergistic effect, where the antibacterial mechanisms of both components work together to exert a stronger inhibitory action against the bacteria. ZnO is known for its intrinsic antibacterial properties, while levofloxacin acts as a potent antibiotic. When combined in nanoparticle form, they can target bacterial cells through different mechanisms, enhancing their overall efficacy.

The sustained release of levofloxacin from the nanoparticle matrix also contributes to its effectiveness. By maintaining a therapeutic concentration of the antibiotic over a prolonged period, ZLFNs can effectively combat bacterial growth and reduce the risk of developing antibiotic resistance.

Overall, the results of this study underscore the potential of ZLFNs as a promising antibacterial agent against *Salmonella typhi*. Their enhanced activity compared to individual components highlights the advantages of using nanoparticle-based formulations in the development of more effective antimicrobial therapies, especially in an era where antibiotic resistance is a growing concern [41].

Conclusions

The advancement of nanotechnology produces new, effective methods to treat a variety of illnesses. These days, a variety of nanoformulations are sold to effectively treat germs resistant to antibiotics. We examine the ZnO-loaded levofloxacin nanoparticles' in vitro release rate, in vitro antioxidant potential, and antibacterial activities both subjectively and quantitatively. Levofloxacin concentration variations were used in experiments to modify the size variations of nanoformulations and the drug entrapment efficiency. Levofloxacin is an antibacterial chemical that can be used to create innovative nanoformulations with potent excipients to improve its water solubility, bioavailability, and antimicrobial activity against a variety of illnesses. This nanoformulations polar phase contains a high-density aqueous solution that includes molecules of surfactant. The use of this unique polymeric nanocarrier containing ZnO-loaded levofloxacin nanoparticles shows promise in the fight against antibiotic resistance *Salmonella typhi*.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

References

1. Du, L. M., Hu, S. J., Chen, X. M., Deng, Y. Y., Yong, H. L., Shi, R. C., ... & Gao, H. J. (2023). Survey of Helicobacter pylori levofloxacin and clarithromycin resistance rates and drug resistance genes in Ningxia, 2020-2022. *Zhonghua yi xue za zhi*, *103*(28), 2163-2167.
2. Garrison, M. W. (2003). Comparative antimicrobial activity of levofloxacin and ciprofloxacin against Streptococcus pneumoniae. *Journal of Antimicrobial Chemotherapy*, *52*(3), 503-506.
3. Cuevas, O., Oteo, J., Lazaro, E., Aracil, B., De Abajo, F., Garcia-Cobos, S., ... & Gallardo, V. (2011). Significant ecological impact on the progression of fluoroquinolone resistance in Escherichia coli with increased community use of moxifloxacin, levofloxacin and amoxicillin/clavulanic acid. *Journal of antimicrobial chemotherapy*, *66*(3), 664-669.
4. Kong, Y., Geng, Z., Jiang, G., Jia, J., Wang, F., Jiang, X., ... & Yu, X. (2023). Comparison of the in vitro antibacterial activity of ofloxacin, levofloxacin, moxifloxacin, sitafloxacin, finafloxacin, and delafloxacin against Mycobacterium tuberculosis strains isolated in China. *Heliyon*, *9*(11).
5. Shen, X., Mu, D., Chen, S., Wu, B., & Wu, F. (2013). Enhanced electrochemical performance of ZnO-loaded/porous carbon composite as anode materials for lithium ion batteries. ACS applied materials & interfaces, 5(8), 3118-3125.
6. Münchow, E. A., Albuquerque, M. T. P., Zero, B., Kamocki, K., Piva, E., Gregory, R. L., & Bottino, M. C. (2015). Development and characterization of novel ZnO-loaded electrospun membranes for periodontal regeneration. Dental materials, 31(9), 1038-1051.
7. Pan, S., Huang, T., Vazan, A., Liang, Z., Liu, C., Wang, J., ... & Sun, J. (2023). Magnesium oxide-water compounds at megabar pressure and implications on planetary interiors. *Nature Communications*, *14*(1), 1165.
8. Liu, R., Luo, C., Pang, Z., Zhang, J., Ruan, S., Wu, M., ... & Gao, H. (2023). Advances of nanoparticles as drug delivery systems for disease diagnosis and treatment. *Chinese chemical letters*, *34*(2), 107518.
9. Ameen, F., Al-Homaidan, A. A., Al-Sabri, A., Almansob, A., & AlNAdhari, S. (2023). Anti-oxidant, anti-fungal and cytotoxic effects of silver nanoparticles synthesized using marine fungus Cladosporium halotolerans. *Applied Nanoscience*, *13*(1), 623-631.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

10. Yao, B., Shi, H., Bi, H., & Zhang, L. (2000). Optical properties of ZnO loaded in mesoporous silica. Journal of Physics: Condensed Matter, 12(28), 6265.
11. Abinaya, S., & Kavitha, H. P. (2023). Magnesium oxide nanoparticles: effective antilarvicidal and antibacterial agents. *ACS omega*, *8*(6), 5225.
12. Sethi, N., Bhardwaj, P., Kumar, S., & Dilbaghi, N. (2019). Development and Evaluation of Ursolic Acid Co-Delivered Tamoxifen Loaded Dammar Gum Nanoparticles to Combat Cancer. *Advanced Science, Engineering and Medicine*, *11*(11), 1115-1124.
13. Bhat, Z. U. H., Hanif, S., Rafi, Z., Alam, M. J., Ahmad, M., & Shakir, M. (2023). New mixed-ligand Zn (II)-based MOF as a nanocarrier platform for improved antibacterial activity of clinically approved drug levofloxacin. *New Journal of Chemistry*, *47*(15), 7416-7424.
14. Sethi, N., Bhardwaj, P., Kumar, S., & Dilbaghi, N. (2019). Development And Evaluation Of Ursolic Acid Loaded Eudragit-E Nanocarrier For Cancer Therapy. *International Journal of Pharmaceutical Research (09752366)*, *11*(2).
15. Agha, O. A., Girgis, G. N., El-Sokkary, M. M., & Soliman, O. A. E. A. (2023). Spanlastic-laden in situ gel as a promising approach for ocular delivery of Levofloxacin: In-vitro characterization, microbiological assessment, corneal permeability and in-vivo study. *International Journal of Pharmaceutics: X*, *6*, 100201.
16. Yusof, N. Y., Norazzman, N. I. I., Zaidi, N. F. M., Azlan, M. M., Ghazali, B., Najib, M. A., ... & Aziah, I. (2022). Prevalence of Antimicrobial Resistance Genes in Salmonella Typhi: A Systematic Review and Meta-Analysis. *Tropical Medicine and Infectious Disease*, *7*(10), 271.
17. Rani, P., & Sethi, N. (2024). To Combat Cancer Cell Lines, the Development, and Evaluation of Lycopene-Co-Loaded Tamoxifen Nanoparticles. Naturalista Campano, 28(1), 1158-1166.
18. Tahir, M. Y., Sillanpaa, M., Almutairi, T. M., Mohammed, A. A., & Ali, S. (2023). Excellent photocatalytic and antibacterial activities of bio-activated carbon decorated magnesium oxide nanoparticles. *Chemosphere*, *312*, 137327.
19. Song, Y., Zheng, C., Li, S., Chen, J., & Jiang, M. (2023). Chitosan-magnesium oxide nanoparticles improve salinity tolerance in rice (Oryza sativa L.). *ACS Applied Materials & Interfaces*, *15*(17), 20649-20660.

20. Saini, A., Budania, L. S., Berwal, A., & Sethi, S. K. N. (2023). Screening of the Anticancer Potential of Lycopene-Loaded Nanoliposomes. *Tuijin Jishu/Journal of Propulsion Technology*, *44*(4), 1372-1383.

21. Saya, L., Malik, V., Gautam, D., Gambhir, G., Singh, W. R., & Hooda, S. (2022). A comprehensive review on recent advances toward sequestration of levofloxacin antibiotic from wastewater. *Science of The Total Environment*, *813*, 152529.

22. Poonam, D., Sethi, N., Pal, M., Kaura, S., & Parle, M. (2014). Optimization of shoot multiplication media for micro propagation of Withania somnifera: an endangered medicinal plant. *Journal of Pharmaceutical and Scientific Innovation (JPSI)*, *3*(4), 340-343.

23. Suman, J., Neeraj, S., Rahul, J., & Sushila, K. (2014). Microbial synthesis of silver nanoparticles by Actinotalea sp. MTCC 10637. *American Journal of Phytomedicine and Clinical Therapeutics*, *2*, 1016-23.

24. Azab, E. T., Thabit, A. K., McKee, S., & Al-Qiraiqiri, A. (2022). Levofloxacin versus clarithromycin for Helicobacter pylori eradication: are 14 day regimens better than 10 day regimens?. *Gut Pathogens*, *14*(1), 24.

25. Sethi, N., Kaura, S., Dilbaghi, N., Parle, M., & Pal, M. (2014). Garlic: A pungent wonder from nature. *International research journal of pharmacy*, *5*(7), 523-529.

26. Ochieng, C., Chen, J. C., Osita, M. P., Katz, L. S., Griswold, T., Omballa, V., ... & Carleton, H. A. (2022). Molecular characterization of circulating Salmonella Typhi strains in an urban informal settlement in Kenya. *PLOS Neglected Tropical Diseases*, *16*(8), e0010704.

27. Jahan, F., Chinni, S. V., Samuggam, S., Reddy, L. V., Solayappan, M., & Su Yin, L. (2022). The complex mechanism of the Salmonella typhi biofilm formation that facilitates pathogenicity: a review. *International Journal of Molecular Sciences*, *23*(12), 6462.

28. Abukhadra, M. R., Gameel Basyouny, M., Khim, J. S., Allam, A. A., Ajarem, J. S., & Maodaa, S. N. (2022). Green functionalization of clinoptilolite with MgO nano-platelets as adsorbent for different species of antibiotic residuals (levofloxacin, ciprofloxacin, and pefloxacin); equilibrium studies. *Separation Science and Technology*, *57*(11), 1688-1701.

29. Chang, L., Xue, X., Deng, Q., Xie, X., Zhang, X., Cheng, C., ... & Huang, Y. (2023). Modulating the electronic structure of Co center via MgO@ C co-doping for PMS

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

activation to remove levofloxacin. *Separation and Purification Technology*, *321*, 124151.

30. Kaura, S., Parle, M., Insa, R., Yadav, B. S., & Sethi, N. (2022). Neuroprotective effect of goat milk. *Small Ruminant Research*, *214*, 106748.

31. da Silva, K. E., Tanmoy, A. M., Pragasam, A. K., Iqbal, J., Sajib, M. S. I., Mutreja, A., ... & Andrews, J. R. (2022). The international and intercontinental spread and expansion of antimicrobial-resistant Salmonella Typhi: a genomic epidemiology study. *The Lancet Microbe*, *3*(8), e567-e577.

32. Rao, D. S., Muraleedharan, K., & Humphreys, C. J. (2010). TEM specimen preparation techniques. *Microscopy: science, technology, applications and education*, *2*, 1232.

33. Mohammed, A., & Abdullah, A. (2018, November). Scanning electron microscopy (SEM): A review. In *Proceedings of the 2018 International Conference on Hydraulics and Pneumatics—HERVEX, Băile Govora, Romania* (Vol. 2018, pp. 7-9).

34. Xue, X., Liao, W., Liu, D., Zhang, X., & Huang, Y. (2023). MgO/Co3O4 composite activated peroxymonosulfate for levofloxacin degradation: Role of surface hydroxyl and oxygen vacancies. *Separation and Purification Technology*, *306*, 122560.

35. Jett, B. D., Hatter, K. L., Huycke, M. M., & Gilmore, M. S. (1997). Simplified agar plate method for quantifying viable bacteria. *Biotechniques*, *23*(4), 648-650.

36. Movasaghi, Z., Rehman, S., & ur Rehman, D. I. (2008). Fourier transform infrared (FTIR) spectroscopy of biological tissues. *Applied Spectroscopy Reviews*, *43*(2), 134-179.

37. D'Souza, S. S., & DeLuca, P. P. (2006). Methods to assess in vitro drug release from injectable polymeric particulate systems. *Pharmaceutical research*, *23*, 460-474.

38. Berthomieu, C., & Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. *Photosynthesis research*, *101*, 157-170.

39. Blanthorne, C., Jones-Farmer, L. A., & Almer, E. D. (2006). Why you should consider SEM: A guide to getting started. In *Advances in accounting behavioral research* (Vol. 9, pp. 179-207). Emerald Group Publishing Limited.

40. Kranz, H., Yilmaz, E., Brazeau, G. A., & Bodmeier, R. (2008). In vitro and in vivo drug release from a novel in situ forming drug delivery system. *Pharmaceutical research*, *25*, 1347-1354.

41. Xu, W., Ding, G., Yokawa, K., Baluška, F., Li, Q. F., Liu, Y., ... & Zhang, J. (2013). An improved agar-plate method for studying root growth and response of Arabidopsis thaliana. *Scientific reports*, *3*(1), 1273.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

Figure 1. PSA image of ZLFNs

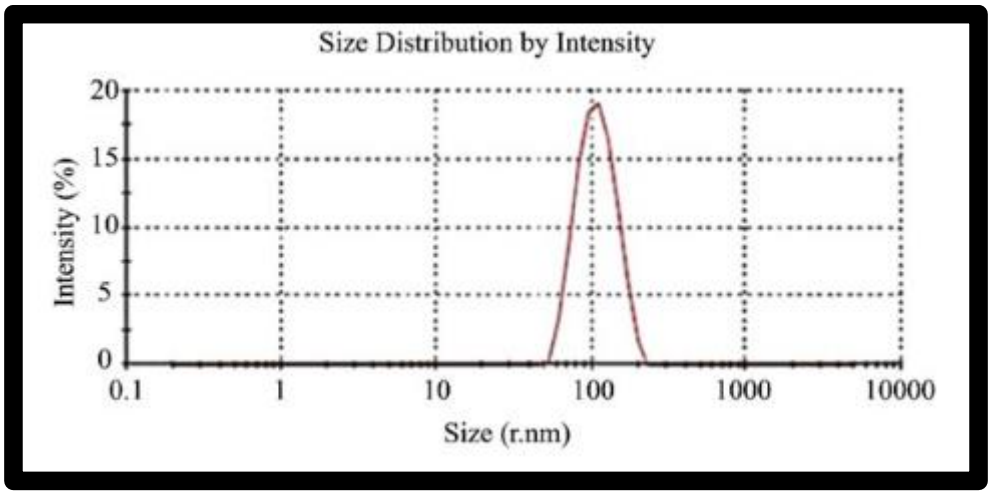


Figure 2. Zeta potential of ZLFNs

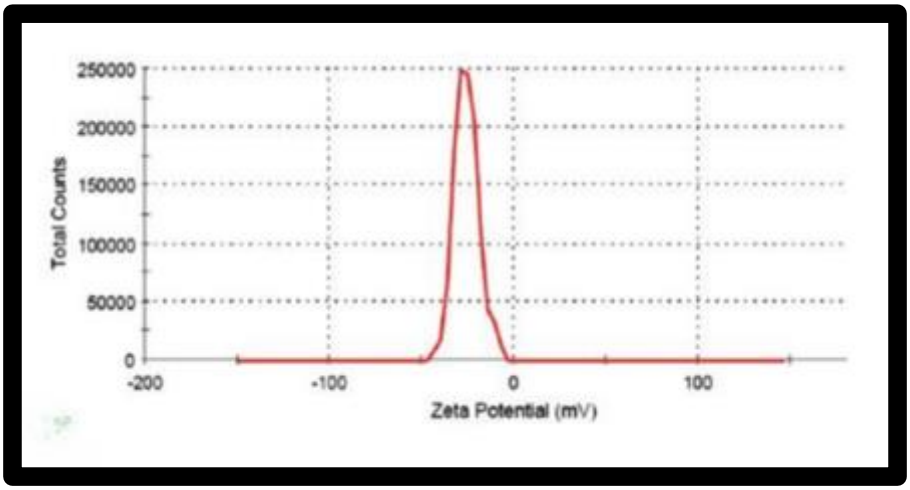
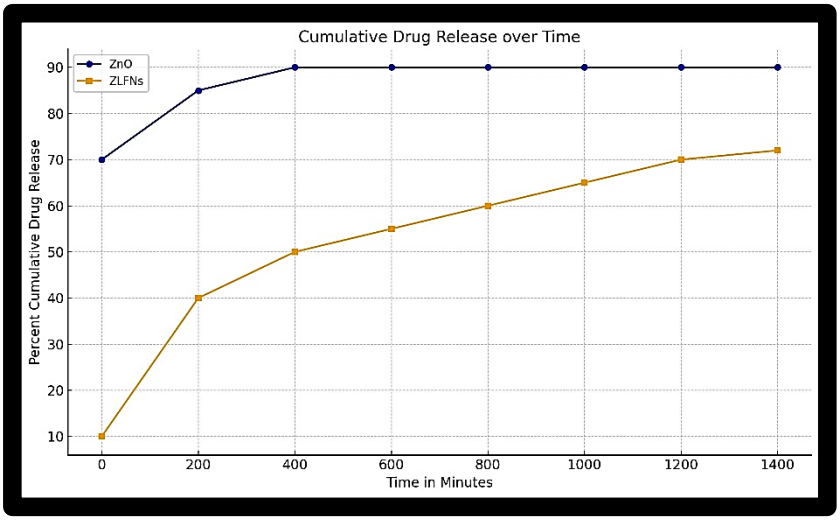


Figure 3. *In vitro* drug release of ZnO and ZLFNs



MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

Figure 4. *TEM* image of ZLFNs

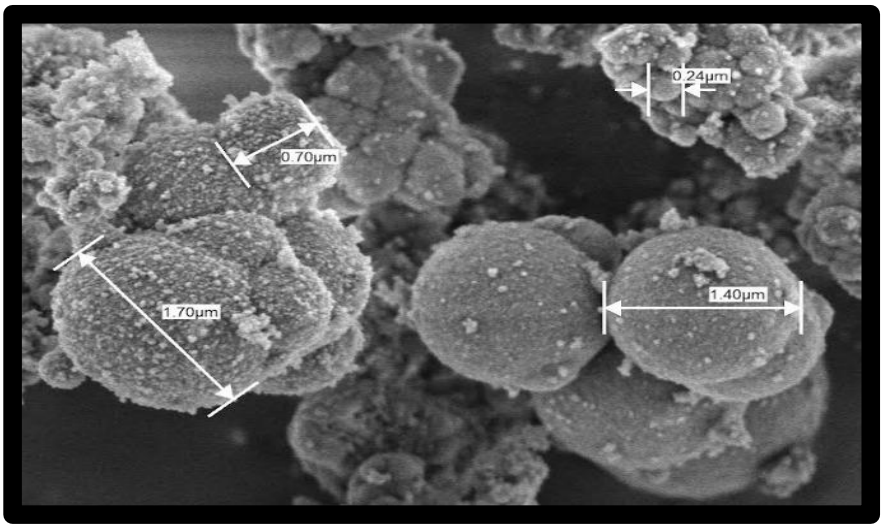


Figure 5. *SEM* image of ZLFNs

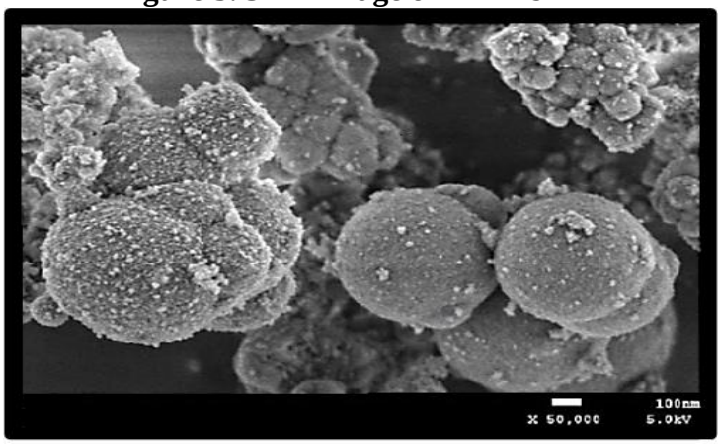
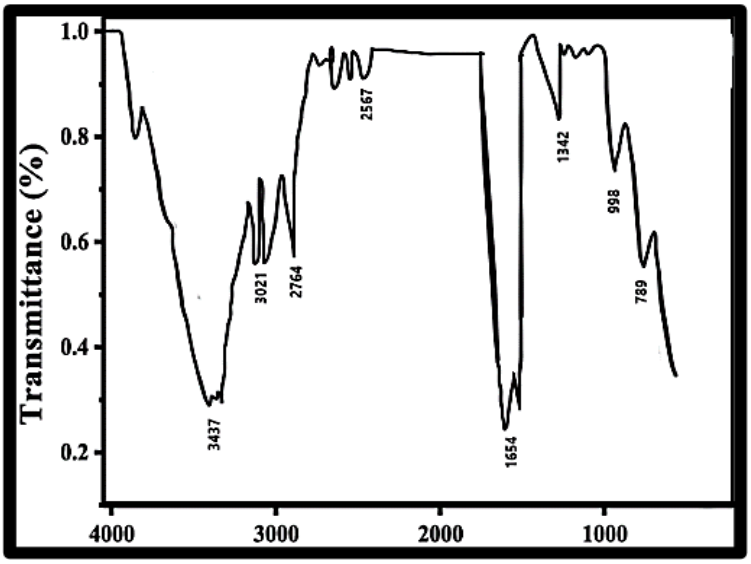


Figure 6. FTIR image of ZLFNs



MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 34 Issue 2, July-Dec 2024, Pages: 412-426

Figure 7. The Antibacterial activity of ZLFNs (A) and levofloxacin (B) against Salmonella typhi.