



Synthesis and Characterization of MgO-loaded Levofloxacin Nanoparticles to Combat Salmonella Typhi bacteria

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ABSTRACT:

A wide variety of ailments, including cancer, autoimmune disorders, and the colonization of bacteria and fungi, are routinely treated with nanomaterials that contain inorganic metallic ions. These nanomaterials are frequently utilized in the treatment of illnesses. This study was conducted to determine whether or not MgO-loaded levofloxacin nanoparticles (MLFNs) might be potentially utilized as an antibacterial agent. To analyze the nanocarriers that were produced through adsorption, instruments such as FTIR, SEM, and TEM were utilized. To conduct in vitro release studies at a temperature of 37 Celsius, simulated body fluid was utilized. After analyzing the data using a variety of kinetic models, it was found that the dissolution was a glacial process that took anywhere from twelve to twenty-four hours to complete. Through the course of study investigations, it was shown that antimicrobial agents have become more effective in combating Salmonella Typhi. Based on the findings of an experimental examination, it was discovered that MLFNs possessed a particle size of 241 nanometers, a zeta potential value of -40.76 millivolts, and a high encapsulation efficacy of 87-91%. These qualities were uncovered as a consequence of the findings of the experiment.

Introduction

When it comes to the domains of biomedical and pharmaceutical technology, the application of nanotechnology is still in its infant stage. For more specific reasons, this is because nanoparticles have a large surface area, increased tissue penetration, protection against drug degradation, and the ability to distribute pharmaceuticals [1-3]. Nanomaterials have the potential to be utilized in a wide range of medical applications, some of which include imaging agents and drug transporters, to name just two particularly noteworthy examples. In the most recent years, cutting-edge drug delivery methods have made use of nanomaterials that are based on carbon, such as nanotubes, graphene, and fullerenes, in addition to nanoparticles that are composed of metal oxides [4-5].

These nanoparticles have been used in a wide range of different applications.

The fluoroquinolone antibiotic known as levofloxacin is capable of exhibiting a wide range of activity against a wide variety of different sorts of infections. This material is often utilized by medical professionals to treat bacterial infections that are present in many parts of the body, including the skin, the urinary tract, the respiratory system, and other areas. It is feasible to stop the process of DNA supercoiling from taking place if one blocks the DNA gyrase enzyme that is found in bacteria. This enzyme is also accountable for preventing DNA replication and repair from taking place. Microorganisms can be eliminated with the use of this method [6]. One of the most important advantages of levofloxacin is that it has a high bioavailability, which indicates that it may be used orally without causing any problems. This is



certainly one of the most significant advantages. In my opinion, this is one of the most appealing features of this antibiotic. Because of its remarkable half-life, it is possible to inject it once daily in many instances. This is because of its effectiveness. Levofloxacin is an antibiotic that is effective in treating bacteria, as was demonstrated in the phrase that came before this one [7].

Magnesium oxide (MgO) has gained considerable interest in recent years due to its antibacterial qualities [8-10]. Magnesium oxide is employed not just in the medical domain but also in the industrial sector. This is becoming a progressively significant aspect in the domain of antimicrobial agents. The antibacterial characteristics of Magnesium oxide stem from its capacity to generate reactive oxygen species (ROS) upon immersion in water. Superoxide ions and hydroxyl radicals are reactive oxygen species (ROS) that cause damage to the DNA, proteins, and lipids of bacterial cells. Oxidative stress is responsible for this damage [11]. Oxidation-induced damage eradicates microorganisms and also impacts crucial bodily systems. Multiple investigations have found that Magnesium oxide is a highly effective antibacterial agent against both Gram-positive and Gram-negative bacteria. An inquiry into the antibacterial effect of coatings for medical devices, wound healing, and water treatment has been conducted [12]. Magnesium oxide nanoparticles have the potential to be effective in combating antibiotic resistance [13-15]. The reason for this is that they have demonstrated encouraging efficacy against antibiotic-resistant bacteria [16]. *Salmonella Typhi*, a group of Gram-negative bacteria, comprises some significant human illnesses as well as harmless commensals and environmental species. The taxonomic order comprises several organisms such as *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella*, and *Yersinia pestis*. *Salmonella Typhi* metabolizes glucose through the Entner-Doudoroff or Embden-Meyerhof-Parnas pathways, resulting in the production of acids and gases [17]. Facultative anaerobes can thrive in both aerobic and anaerobic settings. *Salmonella Typhi* is responsible for causing infections in the gastrointestinal, urinary tract, respiratory, and systemic systems, including sepsis, which makes them significant from a clinical perspective. Certain species within this group exhibit resistance to many antibiotics, hence complicating clinical treatment and exacerbating global antibiotic resistance. *Salmonella Typhi*, albeit virulent, is

a bacterium responsible for causing typhoid fever, which is a potentially fatal disease [18]. This virus is mainly spread through the ingestion of contaminated food or water, particularly in areas with inadequate sanitation and hygiene measures. After being consumed, *Salmonella Typhi* establishes itself in the intestines, where it can reproduce and disseminate throughout the body through circulation, causing a widespread illness. Typhoid fever presents with a range of symptoms, typically including persistent high body temperature, fatigue, abdominal discomfort, cephalalgia, reduced desire to eat, and occasionally a skin eruption. Failure to receive treatment can lead to serious consequences, including intestinal perforation, severe dehydration, and even fatal outcomes. The diagnosis of typhoid fever usually entails doing blood cultures or other laboratory tests to detect the presence of *Salmonella Typhi*. The standard approach for treating typhoid fever often entails the use of medicines to eradicate the bacteria from the body [19]. Nevertheless, the rise of antibiotic resistance, namely towards widely prescribed medications such as fluoroquinolones and third-generation cephalosporins, has emerged as an escalating issue in recent times, hence complicating the process of treatment. The prevention of *Salmonella Typhi* infection is mostly dependent on sanitary measures, including the provision of clean water, appropriate sewage disposal, and the implementation of safe food handling standards. Immunization against typhoid fever is also accessible and advised for persons who are traveling to regions where the illness is prevalent or for those who are at a heightened risk of being exposed. *Salmonella Typhi* continues to be a major public health issue, especially in developing nations. This emphasizes the need for ongoing efforts in both preventing and treating the disease to restrict its transmission and lessen the impact of typhoid fever on affected communities [20].

Materials and Methods

Materials: We procured MgO from a French company named MP Biomedicals, LLC. The source of levofloxacin was sourced from Sigma Aldrich India. The investigation utilized materials of the analytical or reagent type. At our request, the MTTC in Chandigarh supplied us with *Salmonella Typhi*.



Preparation of the MgO-loaded Levofloxacin

A combination of 120 mg of MgO and 250 mg of levofloxacin was mixed in 8 ml of sterile water. After subjecting the mixtures to 40 minutes of ultrasonic oscillations, they were thoroughly cleaned with deionized water many times. Subsequently, they were freeze-dried for the duration of an entire night. Finally, we generated and assessed antibacterial systems that consist of MgO and Levofloxacin utilizing PSA together with dynamic light scattering [28].

Characterization of synthesized MgO-loaded Levofloxacin.

Numerous MLFN properties were examined in the study, including in-vitro drug release, morphology, zeta potential, particle size, entrapment efficiency, and Fourier transform infrared spectroscopy [29].

Particle size: The dynamic light scattering (DLS) technique was used to determine the size distribution (polydispersity index) and average size of the nanoparticles in the nanoformulations. At 25 °C, the mean particle size of the MLFNs was measured using the Zetasizer Nano ZS [30]. The amount of medication in a collected supernatant was quantified using HPLC to measure the drug loading and drug entrapment efficiency [31].

Morphology: To evaluate the enhanced batch's physical structure, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used. The technique known as transmission electron microscopy (TEM) makes it possible to see minute details in materials. Transmission electron microscopy (TEM), in contrast to optical microscopes that use visible light, can reveal astounding detail at the atomic level. This is accomplished by increasing objects up to a fifty million-fold magnification at the nanoscale. The chemical makeup and microscopic structure of a wide variety of materials are regularly investigated using the scanning electron microscope (SEM) [32].

Powdered materials such as MgO, Levofloxacin, and MLFNs were subjected to FTIR spectroscopy employing KBr pellets in the 4500–500 cm⁻¹ range. This work was accomplished by the use of a Fourier transform infrared spectrophotometer [33].

The release profile of MLFNs in vitro: HPLC was used to measure the amount of MgO released from the NPs in vitro. The dialysis sac approach was utilized to investigate the release kinetics. In summary, 10 ml of 0.1 M (pH 7.4) water was used to dissolve 10 mg of magnesium oxide and levofloxacin nanoparticles coated with magnesium oxide. After that, they were put in a dialysis bag together with 250 ml of phosphate buffer saline. A thermostat was used to continuously mix the release material at 80 revolutions per minute. At intervals of 0, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42, and 48 hours, one ml of the sample was taken. Using standard curves at 262 nm, the samples were examined to investigate the release of Levofloxacin nanoparticles loaded with MgO [34].

Antibacterial activity

The Agar well diffusion method will be used to assess the effectiveness against *Salmonella Typhi*. A volume of the microbial inoculum was applied to the whole surface of the agar plate to initiate the inoculation process. Next, an aseptic hole with a diameter of 6 to 8 mm was made using a sterile cork borer or tip. A solution containing the extract or antimicrobial agent was then applied to the well, with a volume ranging from 20 to 100 µL. The agar plates were to be put in an incubator that was appropriate for the bacterium being studied after that. The antimicrobial material stops the microbial strain under investigation from growing as it seeps into the agar medium [35].

Results and Discussion

Particle size and Zeta potential

Particle size and zeta potential were measured on MLFNs. Nanoparticles were 241 nm (Fig 1). Figure 2 shows that AOFNs had a zeta potential of -40.76mV, indicating stable nanoformulations.

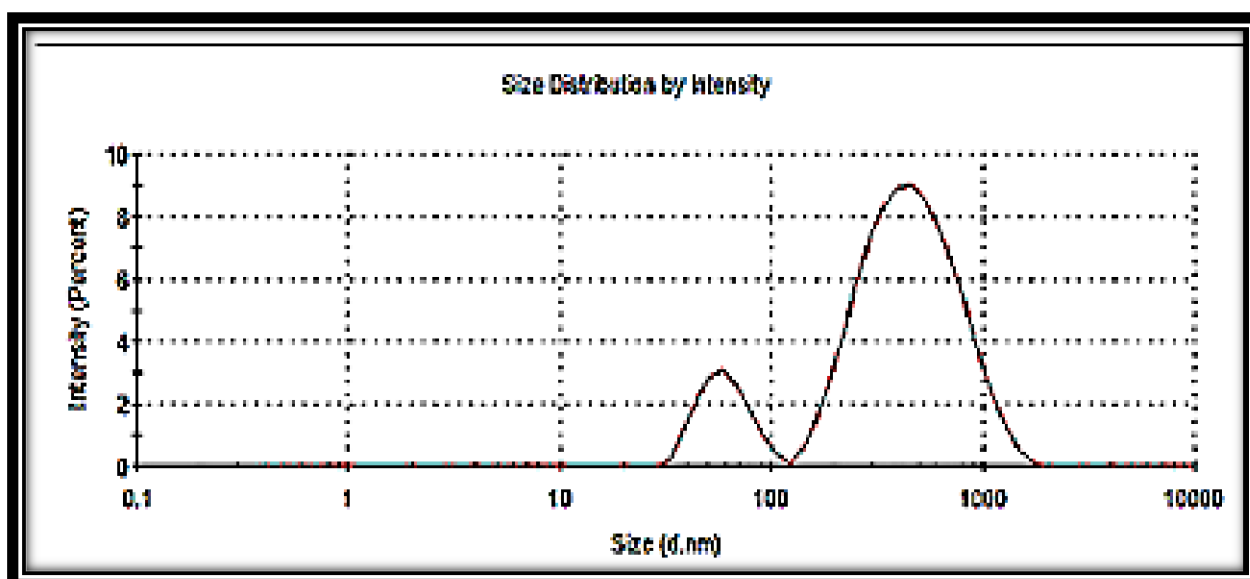


Figure 1: PSA of Nanoparticles

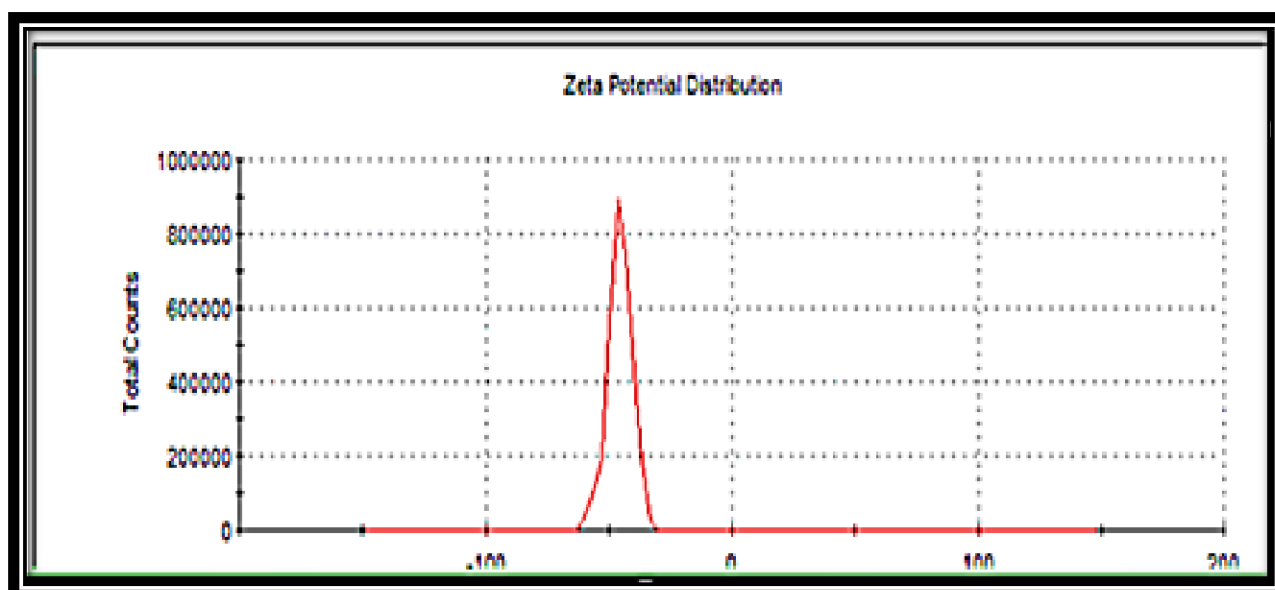


Figure 2: Zeta Potential of Nanoparticles

In vitro release profile of MLFNs

The medication can be released at a controlled rate thanks to the polymeric matrix of the nanoparticles, which also shields it against quick metabolism and degradation. 84.44% of the pure MgO was allegedly released in three hours, based on the in-vitro drug release statistics. MLFNs, on the other hand, showed a constant

release, releasing 38.22% of their MgO after three hours and 72.54% after 24 hours. In general, the hydrophobic (nonpolar) characteristic of MgO is responsible for the drug release of MLFNs, which is a slow release of magnesium over time. Furthermore, levofloxacin produced a cage-like, thick-walled matrix that guaranteed the continuous release of MgO particles.

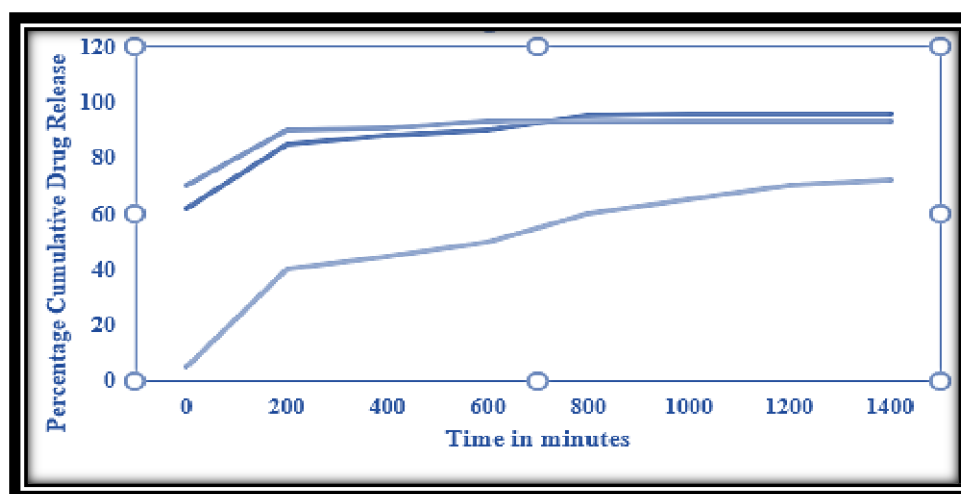
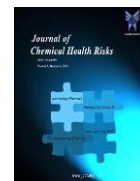


Figure 3: In vitro release Profile

Percentage encapsulation efficiency

The encapsulation efficiency is dependent on the degree of polarity of the molecule, the kind of encapsulating material and media utilized, and the molecular composition of the encapsulating materials. The encapsulation effectiveness for MLFNs varied from 87-91%.

Morphological characterization of MLFNs by TEM, and SEM

The solubility, dissolving rate, and rate of drug release of a nanoparticle is influenced by its size, shape, and dimensions [36]. Nanoparticles can disperse to different

regions of the body based on their size, shape, and dimensions [37-38]. Figure 4 illustrates that the MLFNs, which are spherical and spaced apart, have a particle size ranging from 28 to 51 nm. The diameters of the nanoparticles were found to be different according to both PSA and TEM studies. The TEM operates based on particle-dimensional principles inside a controlled environment, while the PSA principle is based on the ionic mobility of particles [39]. The nanoparticles were proven to be spherical using a scanning electron microscope (SEM) analysis (Fig 5).

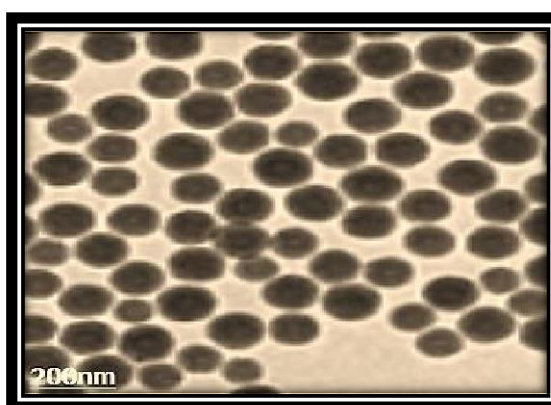


Figure 4: TEM Image of nanoparticles

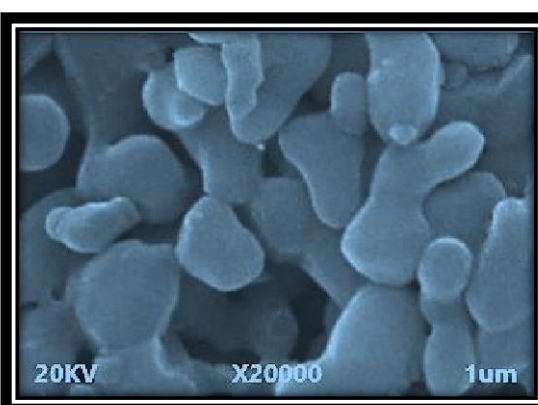


Figure 5: SEM image of nanoparticles

FTIR Analysis of Drug Samples

In order to verify the presence of MgO in levofloxacin nanoparticles (MLFNs) and to determine the outcomes of the interactions between these nanoparticles and levofloxacin, FTIR spectroscopy was employed [30].

Figure 6 demonstrates that the FTIR spectra include intermolecular H-bonding absorption bands at 3478 cm^{-1} and stretching bonds at 2448 cm^{-1} , which are associated with the terminal $-\text{CH}_3$ groups. Figure 7 displays the FTIR spectrum of levofloxacin. The



observation of distinct wave numbers, 1143 cm^{-1} and 765 cm^{-1} , in MLFNs is explained by the presence of weak Van der Waals forces, which are influenced by the mass of the molecules, and dipole-dipole interactions between the molecules. The FTIR spectra of levofloxacin

and MgO exhibit prominent peaks [40]. Although there was a reduction in the highest level of intensity, the bands remained unchanged. There is no chemical bond between MgO and levofloxacin.

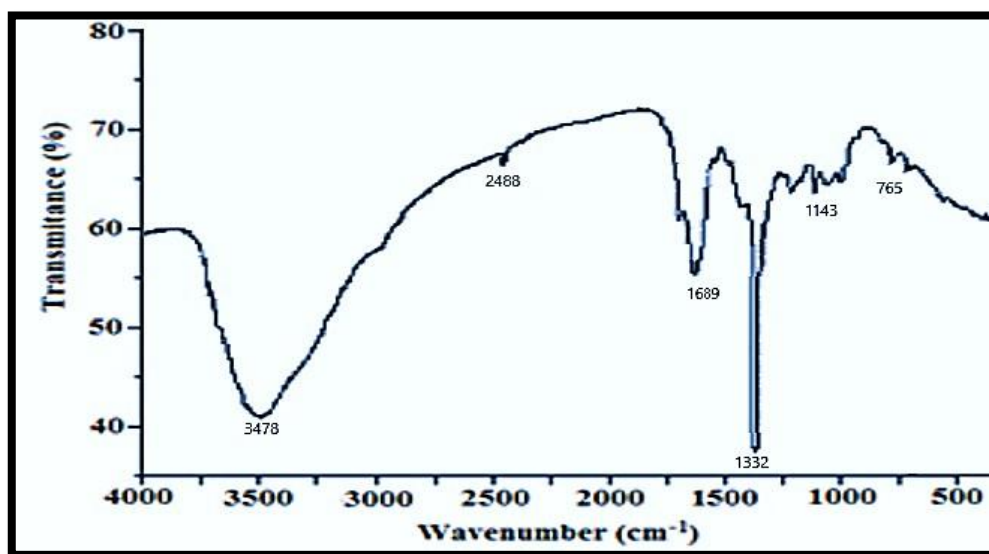


Figure 6: FTIR Spectra of nanoparticles

Antibacterial Activity

Figure 8 displays the outcomes of the antibacterial efficacy of MLFNs when tested against *Salmonella typhi*. MLFNs exhibit much greater efficacy in killing

compared to the control medicines MgO and Levofloxacin. The nanoparticles' substantial surface area may boost their antibacterial effectiveness against *Salmonella Typhi* [41].

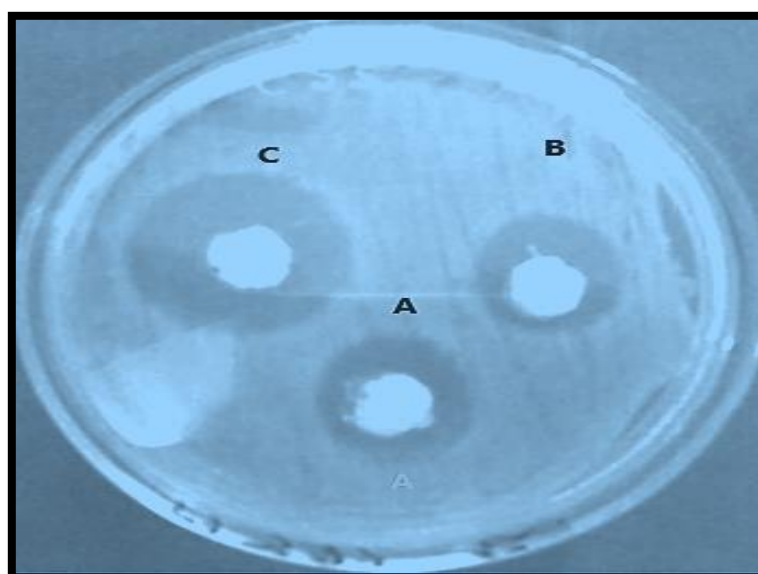


Figure 7: Antibacterial activity of A) MgO B) levofloxacin C) MgO nanoparticles against *Salmonella typhi*



Conclusions

Recent progress in nanotechnology has led to the development of innovative and potent therapies for a wide range of ailments. There are currently multiple nanoformulations that can combat antibiotic-resistant bacteria and other pathogens. We quantitatively and qualitatively assess the antibacterial activity, in vitro release rate, antioxidant capabilities, and levofloxacin nanoparticles loaded with MgO. The researchers conducted experiments with varying quantities of levofloxacin to assess its impact on the drug entrapment efficiency and size changes of the nanoformulations. Levofloxacin, an antibiotic compound, can be utilised to develop novel nanoformulations with powerful additives, resulting in enhanced bioavailability, water solubility, and antimicrobial effectiveness against a wide range of ailments. This polar nanophase consists of a high-density water solution that contains a surfactant. This novel polymeric nanocarrier contains Ofloxacin nanoparticles that are loaded with MgO. It exhibits great potential in combating antibiotic-resistant Salmonella Typhi bacteria.

To effectively prevent Salmonella Typhi infection, immunization alone is insufficient. It is necessary to also implement additional preventive measures such as the use of clean water, sanitation, and healthy hygiene practices [21]. It is highly recommended for anyone who is planning to travel to areas with a high prevalence of Salmonella Typhi to receive vaccination against the disease [22]. Salmonella Typhi, a diverse group of Gram-negative bacteria encompassing both benign commensals and hazardous species, are mostly immunized against certain illnesses. Salmonella, Shigella, Escherichia coli, and Klebsiella pneumoniae possess vaccinations. Salmonella can be prevented through the use of the Vi capsular polysaccharide vaccine and the Ty21a live attenuated oral vaccine. On the other hand, vaccines for Shigella are now under development. Researchers are currently developing vaccinations that target harmful strains of Escherichia coli and Klebsiella pneumonia. These vaccines aim to stimulate the immune system to produce a protective response against specific surface antigens and virulence factors [23]. While there is no universally effective vaccine for Salmonella Typhi, tailored vaccinations can help prevent infections and decrease the overall burden of the disease. To prevent and manage the condition, a range of strategies are

employed, such as administering antibiotics, ensuring a hygienic atmosphere, and receiving vaccinations [24]. In this study, we utilize Magnesium oxide (MgO-loaded levofloxacin (MLFNs) nanoparticles as antibacterial agents on multi-walled carbon nanotube nanotubes. The nanoparticles were analyzed using transmission electron microscopy (TEM), the zeta sizer, and Fourier transform infrared spectroscopy (FTIR) [25-27]. The drug loading and encapsulation of MgO-loaded levofloxacin nanoparticles (MLFNs) were evaluated using high-performance liquid chromatography (HPLC). This evaluation considered the accumulation and display of the pharmacological release profile.

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