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Synthesis and Characterization of Al2O3-Loaded Ofloxacin nanoparticles to Combat Salmonella Typhi Bacteria

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KEYWORDS

Ofloxacin; Toxicity; Antimicrobial; Salmonella Typhi, Antibiotic resistance.

ABSTRACT

Nanomaterials that include inorganic metallic ions are frequently used in the treatment of a wide range of diseases, including cancer, autoimmune disorders, and the colonization of bacteria and fungi. The purpose of this research was to investigate the usage of Al_2O_3-loaded ofloxacin nanoparticles (AOFNs) as an antibacterial agent. FTIR, SEM and TEM, were utilized to investigate the nanocarriers that were created via adsorption. When conducting in vitro release tests at 37 ˚C, simulated bodily fluid was utilized. When the data were analyzed with some different kinetic models, it was discovered that the dissolution was slow and took between 12 and 24 hours. An increase in the effectiveness of antimicrobial agents against Salmonella Typhi was discovered through research investigations. An experimental investigation revealed that AOFNs had a particle size of 233 nm, a zeta potential value of -41.98 mV, and a good encapsulation effectiveness of 89-93%. These characteristics were discovered by the results of the experiment.

Introduction

The application of nanotechnology is still in its infancy within the fields of biomedical and pharmaceutical technology. Specifically, this is because nanoparticles possess a large surface area, greater tissue penetration, protection against drug degradation, and the capability to distribute pharmaceuticals [1-3]. Nanomaterials have the potential to be utilized in a variety of medical applications, including imaging agents and drug transporters, to name just two notable examples. Carbon-based nanomaterials, such as nanotubes, graphene, and fullerenes, as well as nanoparticles comprised of metal oxides, have been utilized in cutting-edge drug delivery systems in recent years [4-5]. These nanomaterials have been used in a variety of applications.

Levofloxacin is a fluoroquinolone antibiotic that exhibits a wide range of activity across a variety of infection types. The treatment of bacterial infections of the skin, urinary tract, respiratory system, and other places is typically accomplished by the utilization of this substance by medical professionals. By blocking the bacterial DNA gyrase enzyme, which is also responsible for interrupting DNA replication and repair, it is possible to prevent the process of DNA supercoiling from occurring. In this way, microorganisms can be eradicated [6]. One of the most significant benefits of levofloxacin is that it has a high bioavailability, which means that it can be taken orally without causing any issues. This is one of my favorite aspects of this antibiotic. In many cases, it is possible to inject it once daily because its half-life is quite impressive. Ofloxacin is an antibiotic that is efficient in treating bacteria, as seen in the previous sentence [7].

One of the reasons that Aluminium oxide (Al_2O_3) has garnered significant attention in recent years is because it exhibits antibacterial properties [8-10]. In addition to its use in the medical field, Aluminium oxide is also utilized in the industrial sector. This is becoming an increasingly important factor in the realm of antimicrobial agents. The ability of Aluminium oxide to produce reactive oxygen species (ROS) when it is immersed in water is the source of the antibacterial properties that it possesses. Superoxide ions and hydroxyl radicals are examples of reactive oxygen species (ROS) that are responsible for causing damage to the DNA, proteins, and lipids of bacterial cells. This

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damage is caused by oxidative stress [11]. Damage caused by oxidation eliminates bacteria, and it also influences the functions that are vital to the functioning of the body. According to the findings of many studies, Aluminium oxide is an efficient antibacterial agent against both Gram-positive and Gram-negative bacteria. As part of the investigation into whether or not it exhibits antibacterial action, coatings for medical devices, wound healing, and water treatment have all been investigated [12]. There is a possibility that Aluminium oxide nanoparticles could be useful in the fight against antibiotic resistance [13-15]. This is because they have shown promising effects against bacteria that are resistant to antibiotics [16]. An order of Gram-negative bacteria called Salmonella Typhi includes numerous major human diseases and many innocuous commensals and environmental species. This taxonomic order includes Escherichia coli, Salmonella, Shigella, Klebsiella, and Yersinia pestis, among others. Salmonella Typhi ferment glucose via the Entner-Doudoroff or Embden-Meyerhof-Parnas pathways, producing acids and gases [17]. Facultative anaerobes may survive aerobic and anaerobic environments. Salmonella Typhi causes gastrointestinal, urinary tract, respiratory, and systemic infections such as sepsis, making them clinically important. Some species in this order are resistant to numerous antibiotics, making clinical treatment difficult and contributing to antibiotic resistance worldwide. Despite their pathogenicity, Salmonella Typhi is a bacterium that causes typhoid fever, a potentially life-threatening illness [18]. This pathogen is primarily transmitted through the consumption of contaminated food or water, often in regions with poor sanitation and hygiene practices. Upon ingestion, Salmonella Typhi colonizes the intestines, where it can multiply and spread throughout the body via the bloodstream, leading to systemic infection. The symptoms of typhoid fever can vary but commonly include sustained high fever, weakness, stomach pain, headache, loss of appetite, and sometimes a rash. If left untreated, severe complications such as intestinal perforation, severe dehydration, and even death can occur. Diagnosis of typhoid fever typically involves blood cultures or other laboratory tests to identify the presence of Salmonella Typhi. Treatment for typhoid fever usually involves antibiotics to eliminate the bacteria from the body [19]. However, antibiotic resistance, particularly to commonly used drugs like fluoroquinolones and third-generation cephalosporins, has become a growing concern in recent years, making treatment more challenging.

Prevention of Salmonella Typhi infection relies heavily on sanitation measures, such as ensuring access to clean water, proper sewage disposal, and safe food handling practices. Vaccination against typhoid fever is also available and recommended for individuals traveling to areas where the disease is endemic or for those at high risk of exposure. Overall, Salmonella Typhi remains a significant public health concern, particularly in developing countries, highlighting the importance of continued efforts in both prevention and treatment strategies to control its spread and reduce the burden of typhoid fever on affected populations [20].

To prevent Salmonella Typhi infection, vaccination alone is not sufficient; additional preventative measures include the utilization of clean water, sanitation, and healthy hygiene practices [21]. Any individual who is going to be traveling to locations where Salmonella Typhi is prevalent is strongly encouraged to get vaccinated against the disease [22]. Salmonella Typhi, a broad order of Gram-negative bacteria that includes harmless commensals and dangerous species, are mostly vaccinated against specific diseases. Salmonella, Shigella, Escherichia coli, and Klebsiella pneumoniae have vaccines. For Salmonella, the Vi capsular polysaccharide vaccine and the Ty21a live attenuated oral vaccine are available, while Shigella vaccines are being developed. Similar vaccines targeting pathogenic Escherichia coli and Klebsiella pneumoniae strains are being developed to induce protective immune responses against surface antigens and virulence factors [23]. There is no universal vaccination for Salmonella Typhi, however, these targeted vaccines prevent infections and reduce disease burden. To prevent and control the disease, various approaches are taken, including the use of antibiotics, maintaining a clean environment, and receiving immunizations [24].

For the goal of this inquiry, Aluminium oxide (Al_2O_3-loaded ofloxacin (AOFNs) nanoparticles are employed as antibacterial agents on multi-walled carbon nanotube nanotubes. To analyze these nanoparticles, transmission electron microscopy (TEM), the zeta sizer, and the Fourier transform infrared spectroscopy (FTIR) were applied [25-27]. Evaluations of drug loading and

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encapsulation of Al_2O_3-loaded ofloxacin nanoparticles (AOFNs) were carried out with the assistance of high-performance liquid chromatography (HPLC). To accumulate and display the pharmacological release profile, this evaluation was taken into consideration.

Materials And Methods

Materials: In France, we purchased Al_2O_3 from a company called MP Biomedicals, LLC. Ofloxacin was obtained from Sigma Aldrich India as the source. For this study effort, the materials that were utilized were of the analytical or reagent sort. The MTTC in Chandigarh provided us with Salmonella Typhi at our request.

Preparation of the Al_2O_3-loaded Ofloxacin

There was a mixture of 135 mg of Al2O3 and 200 mg of Ofloxacin, both of which were combined in 6 ml of sterile water. Following a period of thirty-five minutes of ultrasonic oscillations, the mixes were rinsed many times with deionized water, and then they were freeze-dried for an entire night. Last but not least, the antibacterial systems containing Al2O3 and Ofloxacin were produced and evaluated using PSA in conjunction with dynamic light scattering [28].

Characterization of synthesized Al_2O_3-loaded Ofloxacin.

All aspects of AOFNs, including particle size, entrapment efficiency, zeta potential, morphology, Fourier transform infrared spectroscopy, and in-vitro drug release, were examined [29].

Particle size: The dynamic light scattering (DLS) technique was utilized to determine the average size of the nanoparticles as well as the size distribution (polydispersity index) of the nanoformulations that were being adjusted. The Zetasizer Nano ZS was utilized to determine the average particle size of the AOFNs at a temperature of 25 ˚C [30].

Drug loading and drug entrapment efficiency: HPLC was used to determine them by assessing the amount of medication that was present in a supernatant that had been collected [31].

Morphology: Using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), the morphology of the improved batch was investigated. TEM, or transmission electron microscopy, is a technique that allows for the observation of the tiniest features in matter. In contrast to optical microscopes, which rely on light in the visible range, transmission electron microscopy (TEM) has the potential to reveal astounding detail at the atomic scale. This is accomplished by magnifying nanoscale objects up to fifty million times. SEM is commonly utilized to investigate the chemistry and microstructure of a wide variety of materials [32].

FTIR-spectroscopy: Powdered materials, including Al2O3, Ofloxacin, and AOFNs, were subjected to FTIR in the range of 4500–500 cm−1 as KBr pellets. This was accomplished by utilizing a Fourier transform infrared spectrophotometer. [33].

In vitro, release profile of AOFNs*:* Under in vitro conditions, the amount of Al2O3 emitted from the NPs was determined by HPLC. By employing the dialysis sac technique, the release kinetics were examined. To summarize, 10 mg of Al2O3 and Ofloxacin nanoparticles loaded with Al2O3 were dissolved in 10 ml of 0.1 M (pH 7.4) water. After that, 250 ml of phosphate buffer saline was added before placing them in a dialysis bag. At 80 revolutions per minute, the release media was constantly mixed using a thermostat. One milliliter of the sample was collected at 0, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42, and 48-hour intervals. The samples were analyzed to study the release of Ofloxacin nanoparticles loaded with Al2O3 using standard curves at 254 nm [34].

Antibacterial activity

To determine the efficacy against Salmonella Typhi, the Agar well diffusion method will be employed. The agar plate was inoculated by applying a volume of the microbial inoculum over its whole surface. Then, a sterile cork borer or tip was utilized to create an aseptic hole with a diameter of 6 to 8 mm. To the well, a solution containing the antimicrobial agent or extract, ranging in volume from 20 to 100 µL, was subsequently added. The next step was to place the agar plates in an incubator that was suitable for the microbe under study. As the antimicrobial substance permeates the agar medium, it inhibits the growth of the microbial strain under study [35].

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Results And Discussion

Particle size and Zeta potential

Particle size and zeta potential were measured on AOFNs. Nanoparticles were 233 nm (Fig 1). Figure 2 shows that AOFNs had a zeta potential of -41.98mV, indicating a stable nanoformulation.

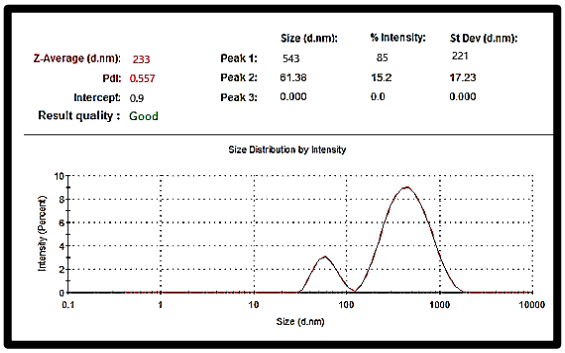

Figure 1: PSA of Nanoparticles

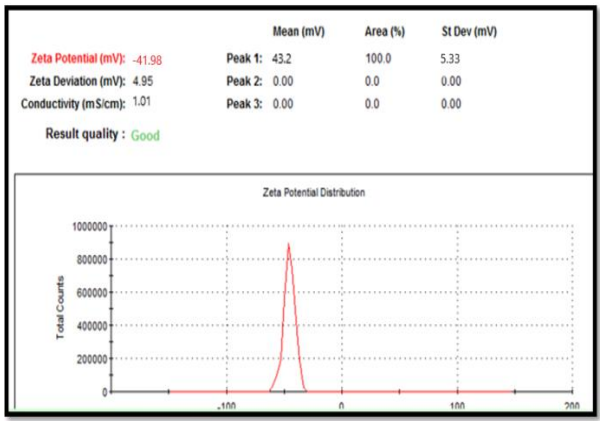

Figure 2: Zeta Potential of Nanoparticles

In vitro release profile of AOFNs

The polymeric matrix of nanoparticles allows for the rate-controlled release of the medicine, protecting it against rapid metabolism and destruction. According to the in-vitro drug release statistics, 84.44% of the pure Al2O3 was supposedly released within three hours. On the other hand, AOFNs demonstrated a continuous release, with 74.02% of their Al2O3 released after 24 hours and 39.66% after 3 hours. In general, the drug release of AOFNs—a gradual release of magnesium over time—is due to Al2O3's hydrophobic (nonpolar) nature. In addition, ofloxacin ensured the Al2O3 particles' continual release by creating a dense matrix with thick walls that resemble a cage.

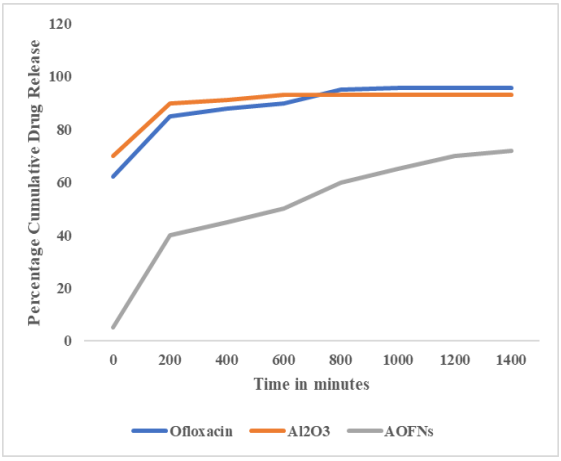

Figure 3: In vitro release Profile

Percentage encapsulation efficiency

The degree of polarity of the molecule, the type of encapsulating material and media used, and the molecular makeup of the encapsulating materials all affect the encapsulation efficiency. For AOFNs, the encapsulation efficiency ranged from 89-93%.

Morphological characterization of AOFNs by TEM, and SEM

A nanoparticle's size, shape, and dimensions affect its solubility, dissolution rate, and rate of drug release [36]. Nanoparticles can migrate to various areas of the body depending on their size, shape, and dimensions [37-38]. Figure 4 shows that the AOFNs, which are spherical and separated, have a particle size range of 28-51 nm. Both PSA and TEM measurements revealed that the nanoparticles' sizes varied. The reason behind this is that the TEM relies on particle-dimensional principles in an isolated atmosphere, but the PSA principle relies on the ionic mobility of particles [39]. Scanning electron microscopy (SEM) investigation confirmed that the nanoparticles were indeed spherical (Fig 5).

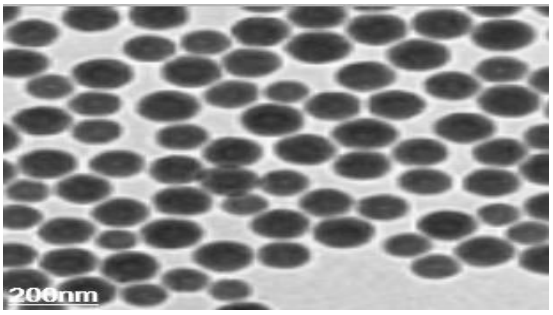

Figure 4: TEM Image of nanoparticles

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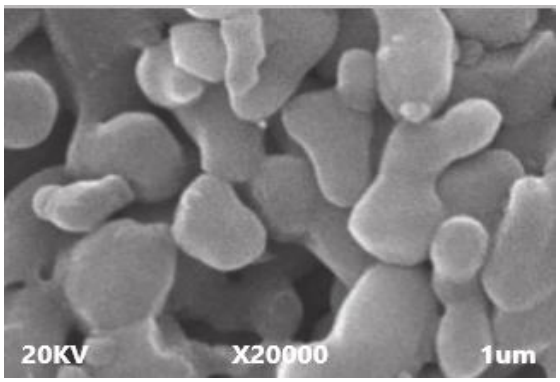

Figure 5: SEM image of nanoparticles

FTIR Analysis of Drug Samples

To confirm that Ofloxacin nanoparticles (AOFNs) were loaded with Al2O3, and to deduce the results of interactions between these nanoparticles and Ofloxacin, FTIR spectroscopy was used [30]. Fig. 6 shows that the FTIR spectra reveal the presence of intermolecular H-bonding absorption bands at 3478 cm^{-1} and stretching bonds at 2448 cm^{-1} which correspond to the terminal -CH_3 groups. This is the ofloxacin FTIR spectrum, as seen in Figure 7. The discovery that wave numbers 1143 cm^{-1} and 765 cm^{-1} differ in AOFNs was attributed to the formation of weak Van der Waals forces, which are mass-dependent, and dipole-dipole interactions between molecules. There are noticeable peaks in the FTIR spectra of Ofloxacin and Al_2O_3 [40]. Despite a decrease in peak intensity, the bands did not change. The absence of a chemical connection between Al_2O_3 and Ofloxacin is indicated.

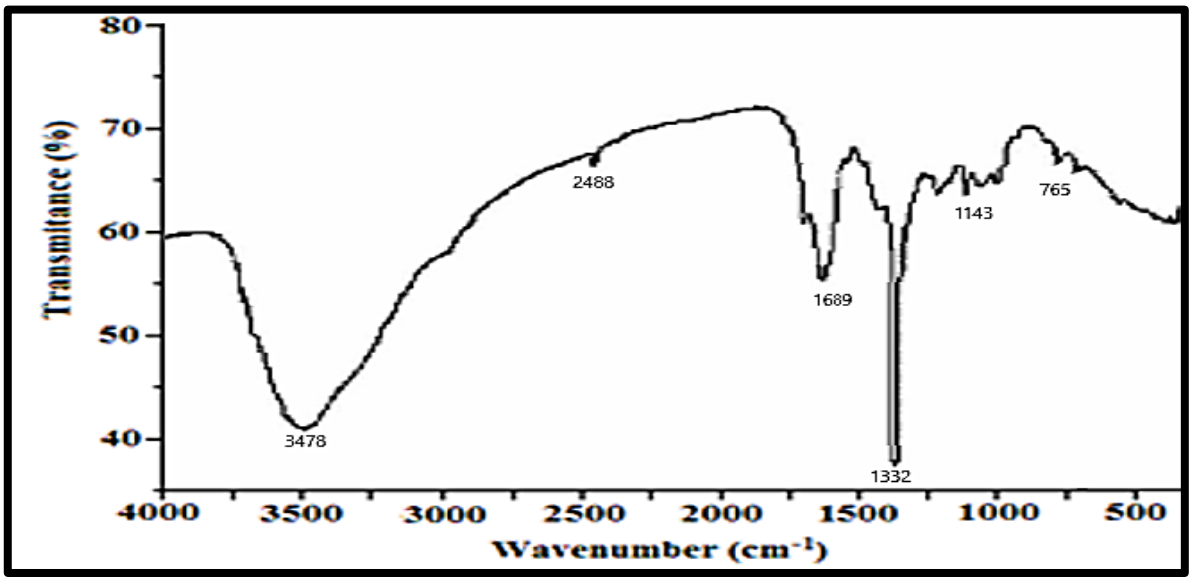

Figure 6: FTIR Spectra of nanoparticles

Antibacterial Activity

Figure 8 shows the results of the antibacterial properties of AOFNs as tested against Salmonella typhi. When compared to the control drugs Al_2O_3 and Ofloxacin, AOFNs have a far higher killing potential. The antibacterial activity of these nanoparticles against Salmonella Typhi may be enhanced by their huge surface area [41].

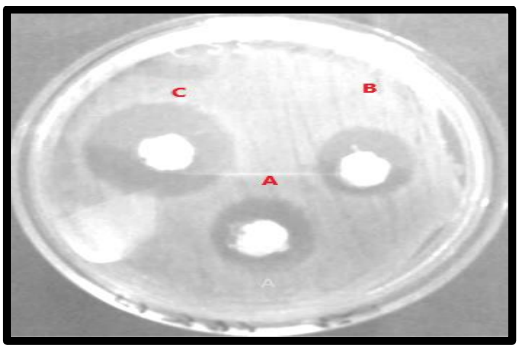

Figure 7: Antibacterial activity of A) Al_2O_3 B) Ofloxacin C) Al_2O_3 nanoparticles

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against *Salmonella typhi*

Conclusions

Thanks to advancements in nanotechnology, new and effective treatments for numerous diseases have been developed. Several nanoformulations are now available to fight antibiotic-resistant bacteria and other microorganisms. We evaluate the antibacterial efficacy, in vitro release rate, antioxidant capacities, and ofloxacin nanoparticles loaded with Al_2O_3 quantitatively and qualitatively. Researchers tested different concentrations of Ofloxacin to see how it affected the drug entrapment efficiency and the size variations of the nanoformulations. The antibacterial chemical ofloxacin can be used to create new nanoformulations with potent excipients, which will increase its bioavailability, water solubility, and antimicrobial efficacy against many diseases. This polar nanophase contains a surfactant-containing high-density water solution. Ofloxacin nanoparticles loaded with Al_2O_3 are contained in this innovative polymeric nanocarrier, which shows tremendous promise in the fight against antibiotic resistance Salmonella Typhi bacteria.

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