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Article

Synthesis and Characterization of Tetracycline Loaded Methionine-Coated NiFe₂O₄ Nanoparticles for Anticancer and Antibacterial Applications

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Abstract: In the present study, nickel ferrite (NiFe₂O₄)-based smart magnetic nanoparticles were fabricated and coated with methionine. Physicochemical characterization of the obtained Met-NiFe₂O₄ nanoparticles revealed the presence of methionine coating over the nanoparticle surface. Drug release study indicated that Tet-Met-NiFe₂O₄ nanoparticles possess pH-responsive controlled drug release behavior for tetracycline (Tet). The drug loading content for Tet was found to be 0.27 mg/L of nanoparticles. In vitro cytotoxicity test showed that the Met-NiFe₂O₄ nanoparticles is biocompatible. Moreover, this magnetic nanostructured material shown strong anticancer property as these nanomaterials significantly reduced the viability of A375 cells when compared to free Tet solution. In addition, Tet-Met-NiFe₂O₄ nanoparticles also showed strong antibacterial activity against different bacterial pathogens.

Keywords: methionine-NiFe₂O₄ nanoparticles; drug release; antibacterial; anticancer; cytotoxicity

1. Introduction

In today's world, hospital-acquired infections caused by viral, bacterial, and fungal pathogens remain the primary concern and biggest challenge among the healthcare workers [1–5]. Antimicrobial molecules include antibiotics and biocides having a bactericidal/bacteriostatic effect on bacteria. Antibiotic is an active substance of synthetic or natural origin which is used to eradicate bacterial infections in humans or animals. While biocide is an active chemical molecule to control the growth of or kill bacteria. Hence, antimicrobial

activity exhibits an inhibitory or lethal effect of a biocidal product or an antibiotic [6–9]. Biocides and antibiotics may share some common behavior and properties in their respective activity and in the resistance mechanisms developed by bacteria. Today, it is important to weigh the risks of selecting antibiotic resistant bacteria by biocide use correctly and to have a clear view of the corresponding emerging health risk. Moreover, understanding the selection and dissemination of biocide resistant pathogens is very important for combating the dissemination of health care associated diseases and foodborne pathogens.

Antibiotics are broadly classified as antibacterial, antifungal, or antiviral drugs depending on their target group [6–8]. For many decades, antibiotics are widely utilized in medical procedures ranging from organ transplants to chemotherapy [10]. From this, the significance of antibiotics is understood as it protects the host cells from microbial or viral infections [11,12]. Towards this, an antibiotic, Tetracycline (Tet), has been extensively used to treat various bacterial infections [13,14]. In addition, Tet is a well-recognized antibiotic used in the treatment of skin cancer and shown to inhibit several other cancer types.

The functional role of antibiotics is mainly based on the drug delivery system and the mechanism of their controlled delivery to targeted affected cells [15–18]. Recently, several organic-inorganic hybrid nanoparticles have been extensively used in anticancer therapy. Indeed, methionine is one of the essential amino acids i.e., required for the human growth due to its antioxidant activity. The activated functional groups of methionine (-COOH and -NH₂) are used to conjugate the metal atoms, and further the loading and release behavior are investigated. The design and synthesis of organic-inorganic hybrid nanoparticles through combining the advantages of both organic and inorganic counterparts may improve the overall properties, such as particle size, surface charge, and many other physicochemical properties [19,20]. Roca et al. (2012) reported that modified magnetic NPs (MNPs) has shown increased efficacy under in vivo conditions [21].

Several recent findings have emphasized the therapeutic role of MNPs in treating the disease [22–24]. Nanoparticles whose particle size ranging between 10–100 nm have shown properties to improve drug bioavailability and facilitate the targeted accumulation of drugs inside the cell through enhanced permeability and retention (EPR) effect [25,26]. Magnetic spinel ferrite nanoparticles are widely used in biotechnology. The main advantage of magnetic nanoparticles is that can be readily isolated from the solution media using an external magnetic field. NiFe₂O₄ nanoparticles have gained worldwide acceptance due to their low coercivity, high saturation magnetization, excellent chemical stability, high Curie temperature, and electromagnetic performance [27]. However, to the authors' best knowledge, the utilization of NiFe₂O₄ magnetic nanoparticles in the field of biomedicine is available. Further, NiFe₂O₄ nanoparticles are widely used as an in vivo magnetic hyperthermia agent in biomedicine [28]. NiFe₂O₄ nanoparticles exhibited an inverse spinel structure with Ni⁺² in octahedral sites (Ni(OH)) and Fe⁺³ equally distributed between tetrahedral (Fe (Td)) and octahedral sites (Fe(OH)) of the O_h²⁻ FCC (Face-Centered Cubic) cell [29]. The complete crystalline structure belongs to O_h⁷ space group with oxygen atoms occupying the 32e positions, Fe (Td) atoms occupying the 8a ones and the Ni(OH) and Fe(OH) atoms are distributed on 16d positions [30]. Different methods, including solvothermal, sol-gel, co-precipitation, microemulsion, and thermal decomposition, have been utilized to fabricate magnetic nanostructures of varying morphologies and structural variants [31–34] which has immense scope and applications in biomedical science.

Various amino acids have been successfully applied in the preparation of magnetic nanoparticles. However, the use of methionine in the fabrication of nickel-ferrite based nanocomposite is not reported yet. Recently, Saykova et al. synthesized magnetic NiFe₂O₄@Au crystal nanoparticles using the amino acid methionine as a reducing and stabilizing agent [35]. The nanoparticles obtained in this study after three stages of gold deposition had an average particle size of about 120 nm, which is relatively large for cellular adsorption studies and for biomedical applications thereof. In this study, methionine-coated nickel ferrite nanoparticles with an average particle size of about 27 nm are synthesized

in a simple and cost-effective step by reflux method, which is the preferred magnetic nanomaterial for various biological applications.

Recently, non-coated nickel ferrite magnetic nanoparticles were synthesized by Majed et al. and tested in a rat model [36]. However, tetracycline loading on this nanostructure (Met-NiFe₂O₄), and its effect on normal and A-375 cancer cells has not been studied. In addition, the loading of tetracycline on Met-NiFe₂O₄ as a biocompatible and targeted nanocarrier and its effect on bacterial growth remain unexplored. Compared to previous literature, it can be concluded that we have prepared nickel ferrite nanoparticles coated with methionine in a facile synthetic route which is explored for anticancer and antibacterial applications.

In the present study, a simple and effective strategy is developed to synthesize Met-NiFe₂O₄ nanoparticles employing a one-step reflux procedure. Various characterization studies are performed to examine the shape, structure, and magnetic characteristics of Met-NiFe₂O₄ nanoparticles. Tet antibiotic is used to explore the drug loading and release profile of Met-NiFe₂O₄ nanoparticles, for the first time. Moreover, MTT tests are used to assess the in vitro cytotoxicity of Met-NiFe₂O₄ nanoparticles before and after Tet loading of varying doses and incubation periods. Furthermore, antibacterial activity of Met-NiFe₂O₄ nanoparticles against pathogenic bacteria is assessed. To date, the cytotoxicity and antibacterial properties of Met-NiFe₂O₄ nanoparticles have not been reported.

2. Materials and Methods

2.1. Materials

Ferric chloride hexahydrate (FeCl₃·6H₂O), Nickel chloride hexahydrate (NiCl₂·6H₂O), sodium hydroxide, methionine, and other chemicals were procured from Merck, Germany. A-375 and HFF cells were obtained from the Pasteur cell bank. *Staphylococcus aureus* (ATCC 23235), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), and *Enterococcus faecalis* (ATCC 29212) strains were collected from The Pasteur Institute of Iran.

2.2. Fabrication of Met-NiFe₂O₄ Nanoparticles

Met-NiFe₂O₄ nanoparticles were synthesized by reflux method (as shown in Figure 1). In brief, 2.431 g of FeCl₃·6H₂O and 1.069 g of NiCl₂·6H₂O were dissolved in 120 mL of distilled water (d.H₂O), followed by the addition of 1.5 M NaOH solution to adjust the pH to 12. Then, a brown colored solution appeared, and was filtered and washed several times with d.H₂O until the pH of the filtrate becomes neutral (7). Next, 1.5 g of methionine was added. The resulting mixture was heated to 70–80 °C and refluxed for 3 h. The final product was collected by strong magnetic separation and rinsed three times with absolute ethanol and d.H₂O. In a similar way, NiFe₂O₄ nanoparticles were synthesized, but without methionine.

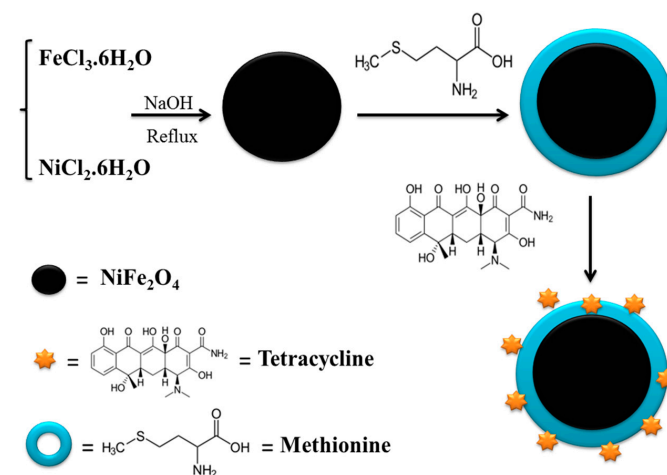


Figure 1. Synthesis and Tet loading on Methionine-coated nickel ferrite nanoparticles (Met-NiFe₂O₄ nanoparticles).

2.3. Physicochemical Characterization Studies

X-ray diffraction (XRD) analysis NiFe₂O₄ and Met-NiFe₂O₄ nanoparticles was carried out using the STOE STADI-P instrument (STOE, & Cie GmbH, Darmstadt, Germany). Field Emission-Scanning Electron Microscopy (FESEM) (Zeiss-EHT-10.00 kV, Carl Zeiss SMT AG Company, Oberkochen, Germany) and High Resolution-Transmission Electron Microscopy (HR-TEM) (Zeiss-EM10C-100 kV, Carl Zeiss SMT AG Company, Oberkochen, Germany) instruments were used to determine morphology and particle size of nanoparticles respectively. The particle size was measured in a dry state through the particle size distribution curve. Next, Fourier Transform Infrared Spectroscopy (FTIR) (Nexus 870, Nicolet, Madison, WI, USA) was also performed. Absorbance measurements at 275 nm through means of UV/visible spectroscopy (Shimadzu UVS-1700, Shimadzu Corporation, Kyoto, Japan) was employed to study the amount of drug adsorbed on nanoparticle surface and the controlled drug release behavior. Thermal properties were analyzed by Thermogravimetric Analyzer (TGA) (Shimadzu TA Q600, Shimadzu TA Instrument SDT Q600, New Castle, DE, USA) in a temperature range of 25 to 800 °C under Nitrogen atmosphere at a constant heating rate. Finally, the magnetic properties were studied by Quantum Design MPMS-XL-7 instrument (MPMS-XL-7), San Diego, CA, USA.

2.4. Average Hydrodynamic Size and Zeta Potential Measurement

The average hydrodynamic size (Z-average) (based on light diffraction), particle size distribution (PDI), and average zeta potential (based on electrophoretic movement of particles) of NiFe₂O₄, Met-NiFe₂O₄ and Tet-Met-NiFe₂O₄ nanoparticles were determined by Malvern Zetasizer 2000 HS instrument (Malvern, UK) where nanoparticles were diluted 100 times in distilled water at 25 ± 0.1 °C prior to experiment.

2.5. Tetracycline Loading and Release Behavior

Various concentrations of Tet were added to 0.2 mg of Met-NiFe₂O₄ nanoparticles and incubated in the dark conditions for 24 h at room temperature. The resulting Tet-Met-NiFe₂O₄ nanoparticles were pelleted using centrifuge, and the collected supernatant was subjected to absorbance measurements at 275 nm using UV/Visible spectroscopy to determine the drug loading efficiency of Tet (Figure 1).

Next, 10 mg of Tet-Met-NiFe₂O₄ nanoparticles was suspended into 10 mL of PBS buffer of pH 5 and 7.4 at 37 °C under dark conditions. Then, 1 mL supernatant was collected at different time intervals and replaced with fresh PBS. Finally, the concentration of released Tet was calculated using UV/Visible spectroscopy (Shimadzu UVS-1700, Shimadzu Corporation, Kyoto, Japan). The drug release data were fitted with different kinetic models' equations for determining the mechanism of drug release from Tet-Met-NiFe₂O₄ nanoformulations. The most appropriate drug release kinetic model was determined by analyzing the regression coefficients of graphs. According to this, the drug release mechanism was determined from the drug delivery system.

2.6. In Vitro Cytotoxicity

A standard MTT test was performed to analyze the in vitro cytotoxicity of Tet-Met-NiFe₂O₄ nanoparticles on A375 and HFF cell lines. These cell lines were cultured using RPMI-1640 fresh medium supplemented with 10% FBS and 1% penicillin/streptomycin in a humid CO₂ incubator with 5% carbon dioxide at 37 °C. Once the cells had achieved 85–95% confluence, the media was aspirated. Detachment of the cell monolayer was performed using trypsin-EDTA (0.25% (*w/v*)). Then, the detached cells were resuspended in a complete growth medium, labeled with trypan blue, and counted using hemocytometer. Different concentrations of Tet-Met-NiFe₂O₄ (0–70 µg/mL) were added to the cultured A375 and HFF cells and incubated for 72 h. For cell proliferation studies, the cells were incubated with 0.5 mg/mL of MTT reagent for 4 h. Then, the purple crystal formazan formed was dissolved in 100 µL of DMSO for the colorimetric determination of the cells' oxidoreductase enzymatic activity. The absorbance values of both control and test concentrations were

measured at 570 nm while reference blank measurements taken at 630 nm, and then the cell viability and IC₅₀ values were calculated [7,15,37]. The percentage of cell viability was calculated using Equation (1).

$$\text{Cell Viability (\%)} = \frac{\text{absorbance of treated cells}}{\text{absorbance of control cells}} \times 100 \quad (1)$$

2.7. Antibacterial Activity

The antibacterial activities of free Tetracycline and Tet-Met-NiFe₂O₄ nanoparticles were evaluated against both Gram-positive and Gram-negative bacteria, such as *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*, using microdilution method. Next, the obtained data was used to calculate the minimum bactericidal concentrations (MBC) and minimum inhibitory concentrations (MIC). Different samples were initially made using Mueller–Hinton Broth (MHB), then 100 µL of each sample was added into 96-well microplates. Approximately 0.5 McFarland standard bacterial suspension was prepared and diluted in Mueller–Hinton Broth to achieve a final concentration of 1×10^6 colony forming units (CFU)/mL. Then, 100 µL of diluted bacterial suspension was mixed with 100 µL of samples (with different concentrations as mentioned above) in 96-well microplates and incubated at 37 °C for 18 h. Bacterial suspension without the drug acts as positive control, while the negative control was the highest drug concentration without bacteria. MBC test was performed to confirm the results of MIC test. Consequently, 100 µL of clear wells with no visible bacterial growth was transferred to petri plates containing MHA (Mueller–Hinton Agar) medium and incubated for overnight at 37 °C [7,8].

2.8. Statistical Analysis

All tests were performed in triplicates ($n = 3$) and each test repeated at least three independent times. The equality of variance and normality were checked by Brown–Forsythe and Shapiro–Wilk tests, respectively. Then, the data was compared using one way or two-way analysis of variance (ANOVA) with repeated measures, followed by Tukey’s or Sidak’s post hoc comparison test. The results were given as mean \pm SD. The differences were considered significant when a p -value of less than 0.05 is considered statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Results and Discussion

3.1. Synthesis and Physicochemical Characterizations of Tet-Met-NiFe₂O₄ Nanoparticles

3.1.1. XRD Analysis

The method of fabrication of Met-NiFe₂O₄ nanoparticles is outlined in Figure 1. XRD analysis showed the diffraction peaks of crystal spinel NiFe₂O₄ nanoparticles at 18.60°, 30.49°, 35.88°, 37.45°, 43.60°, 53.98°, 57.54°, and 63.12° 2θ values (JCPDS No. 98-006-0930) while in the case of Met-NiFe₂O₄ nanoparticles, the diffraction peaks were observed at 18.57°, 30.24°, 35.73°, 37.44°, 43.32°, 53.69°, 57.28°, and 62.77° 2θ values (JCPDS No. 98-006-0930) (Figure 2A). The entry of methionine into the network cavity and an increase in network space is reflected through a drop in 2θ value corresponding to crystal spinel NiFe₂O₄ nanoparticles. The characteristic diffraction peaks for two samples are indexed to the crystal planes of (111), (220), (311), (222), (400), (422), (511), and (440). Met-NiFe₂O₄ nanoparticles with average size of 27 nm as calculated from the full-width value at half maximum (FWHM) of broadened characteristic peaks using Debye–Scherrer’s Equation (2).

$$D = \frac{0.9\lambda}{\text{FWHM} \cos \theta} \quad (2)$$

where D denotes the crystalline size, β is the full width at half maximum (FWHM), θ represents the Bragg angle corresponding to the peak, and λ is the wavelength of the X-rays.

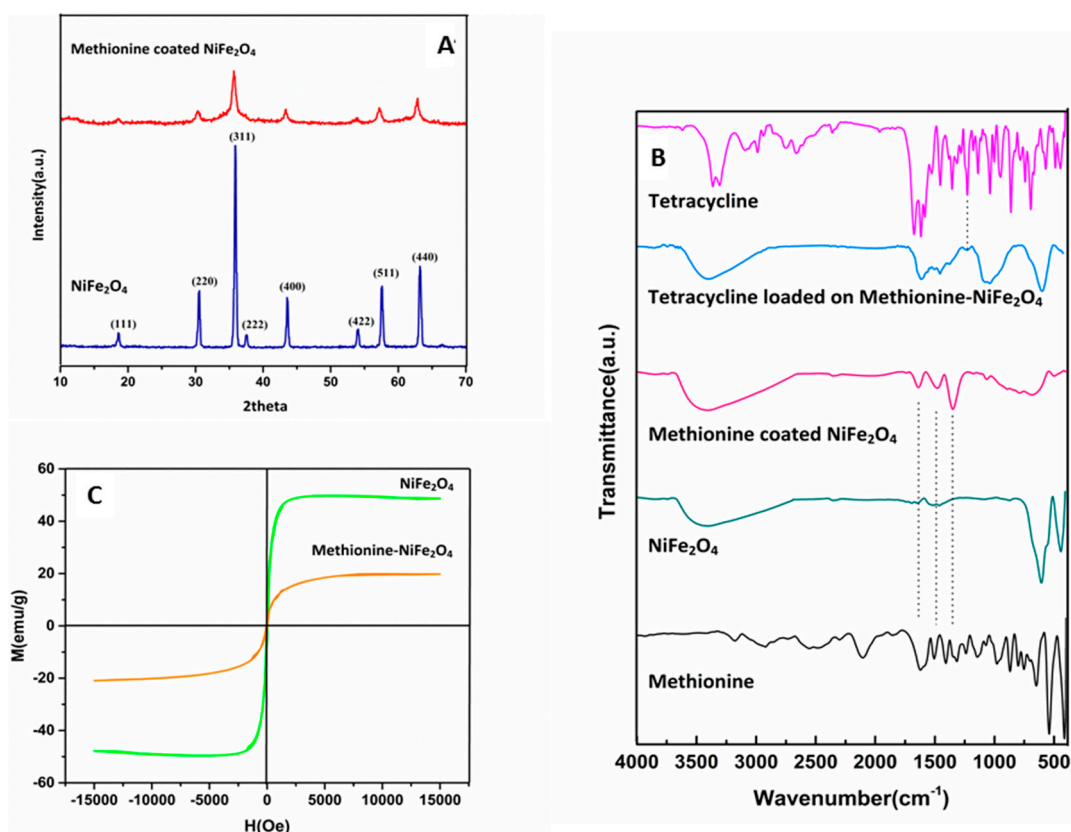


Figure 2. X-ray diffraction patterns of NiFe₂O₄ nanoparticles with and without methionine coating (A), FTIR spectrum of methionine, Tet, NiFe₂O₄ nanoparticles, Met-NiFe₂O₄ nanoparticles, Tet-Met-NiFe₂O₄ nanoparticles, (B) and Magnetization curves of NiFe₂O₄ and Met-NiFe₂O₄ nanoparticles (C).

3.1.2. FT-IR Spectral Analysis

FTIR analysis was used to study the nature of functional groups present on the surface of MNPs. The FTIR spectra of methionine, free Tet, bare NiFe₂O₄, Met-NiFe₂O₄ nanoparticles, and Tet-Met-NiFe₂O₄ nanoparticles are shown in Figure 2B. The Methionine amino acid spectrum is a combination of carboxylate salts and the first type amine. The symmetric and asymmetric N-H bending assigned in the region at 1517 cm⁻¹ and 1630 cm⁻¹ respectively. Furthermore, the symmetric and asymmetrical stretching of the COO⁻ bond was exhibited at 1419 cm⁻¹ and 1600 cm⁻¹, respectively [38]. C-O bond represented at 1232–1330 cm⁻¹. The inherent vibration of tetrahedral and octahedral metal-oxygen complexes of NiFe₂O₄ is ascribed to regions observed at 400 and 600 cm⁻¹, respectively, which are mostly dependent on Fe-O distance [38].

FTIR spectrum of Met-NiFe₂O₄ nanoparticles revealed that methionine was present on the surface of NiFe₂O₄ nanoparticles. Tet features two small vibration at 500–569 cm⁻¹ in its FT-IR spectra, which is linked to out-of-plane ring deformation. The regions at 1000–1257 cm⁻¹ are associated with C-H in-plane deformation vibrations, while those at 998 cm⁻¹ are associated with C-N stretching. Two bands appeared at 1462 and 1366 cm⁻¹ displaying the bending vibrations of C-H and CH₃ groups, respectively. Band of C=C stretching is observed at 1571–1632 cm⁻¹. C-H and CH₃ stretching are attributed to the vibrational at 3013–3078 cm⁻¹ and 2819–2949 cm⁻¹. Bands of N-H and O-H stretching is ascribed to the regions which seen at 3319–3340 cm⁻¹ [39]. After Tet loading, the regions at 998–1257 cm⁻¹ were originated in the FTIR spectrum, which is most likely due to C-N stretching and C-H vibration bands in-plane deformation of Tet. These findings supported that methionine's gets effectively coated onto MNPs, and, thus, possess good potential for drug loading and acts as smart nanocarrier for drug delivery.

3.1.3. Magnetic Property Analysis

Figure 2C displayed the magnetic properties of bare NiFe_2O_4 and Met- NiFe_2O_4 nanoparticles at room temperature in a magnetic field ranging from -15 kOe to 15 kOe. These MNPs exhibited the superparamagnetic behavior. The saturation magnetization (M_s) of bare NiFe_2O_4 and Met- NiFe_2O_4 nanoparticles were calculated to be 47.56 and 19.80 emu/g, respectively. Further, the saturation magnetization (M_s) of Met- NiFe_2O_4 nanoparticles was also reduced at room temperature. Superparamagnetic behavior is an important feature for biomaterials, which facilitate their tracking in the magnetic field while keeping the advantage of a stable and homogeneous suspension during drug delivery.

3.1.4. Size and Morphology Analysis

FE-SEM (Figure 3A) and HR-TEM (Figure 3B,C) images indicated that NiFe_2O_4 nanoparticles, Met- NiFe_2O_4 nanoparticles, and Tet-Met- NiFe_2O_4 nanoparticles, were found to be spherical in shape and possess nearly uniform sizes. Besides this, the TEM images of Met- NiFe_2O_4 and Tet-Met- NiFe_2O_4 nanoparticles showed a mild aggregation, probably due to the strong magnetic interactions between the NiFe_2O_4 nanoparticles. Methionine was successfully coated onto the surface of NiFe_2O_4 nanoparticles, and the particle size was calculated to be 28 – 29 nm.

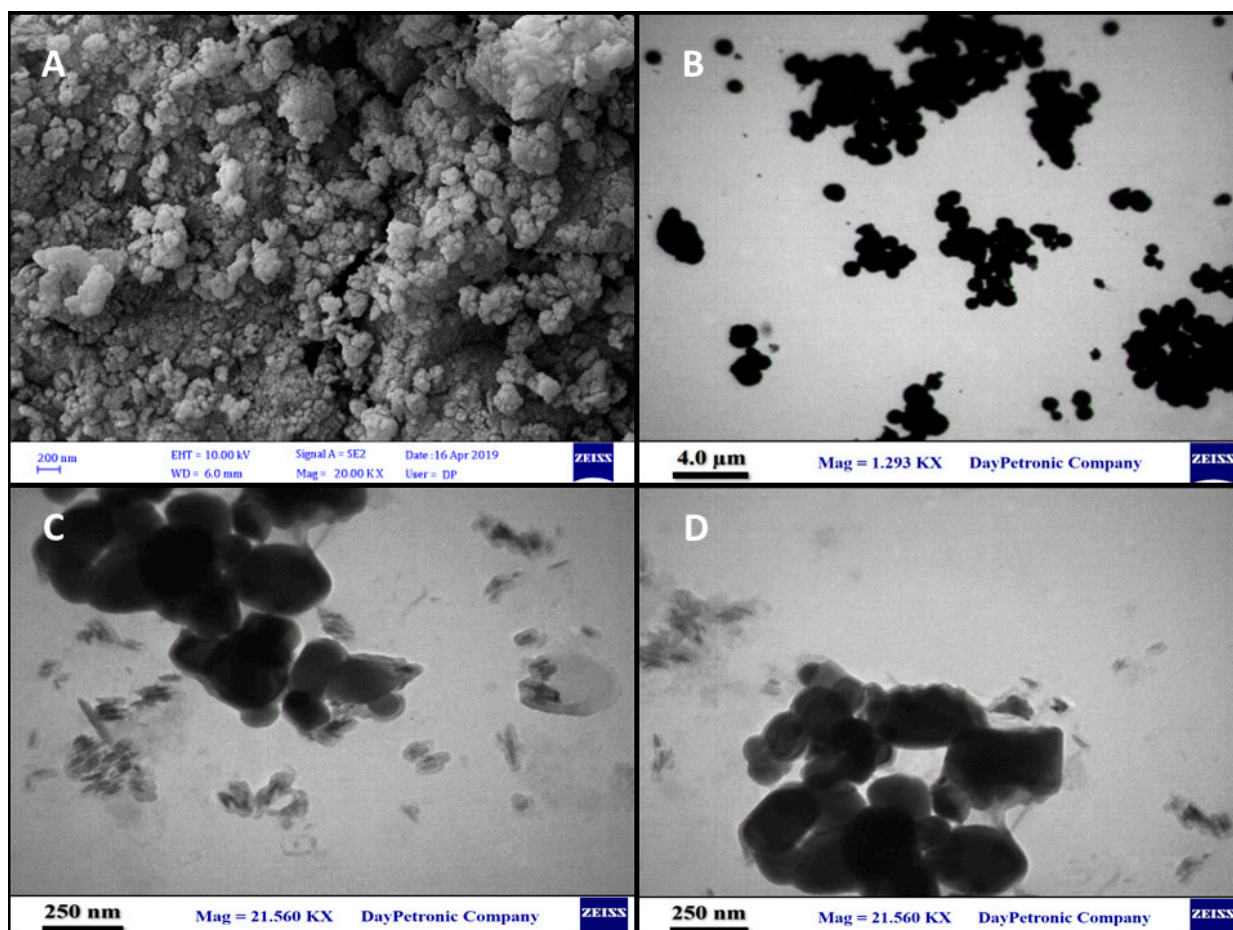


Figure 3. FE-SEM of Met- NiFe_2O_4 nanoparticles (A), TEM images of NiFe_2O_4 nanoparticles (B), Met- NiFe_2O_4 nanoparticles (C), and Tet-Met- NiFe_2O_4 nanoparticles (D).

3.1.5. Thermogravimetric Analysis

Thermogravimetric analysis (TGA) of methionine, NiFe_2O_4 nanoparticles, and Met- NiFe_2O_4 nanoparticles (Figure 4) were recorded. TGA profile showed that the initial weight loss observed at 150 to 260 $^\circ\text{C}$ could be due to thermal decomposition of carbon

dioxide, water and fine molecules trapped between the layers. On the other hand, heat-induced thermal degradation of methionine could be noticed at 260–300 °C, which is also consistent with TGA profile of amino acid, methionine. Further increase in temperature above 350 °C showed no change in weight which could be due to the high stability of the nanostructure [33,40]. The weight loss observed near 300 °C for NiFe₂O₄ nanoparticles and Met-NiFe₂O₄ nanoparticles were found to be 8.3% and 13.8%, respectively. Difference in the weight loss between NiFe₂O₄ nanoparticles and Met-NiFe₂O₄ nanoparticles near 300 °C indicated that methionine was successfully coated onto the surface of the NiFe₂O₄ nanoparticles.

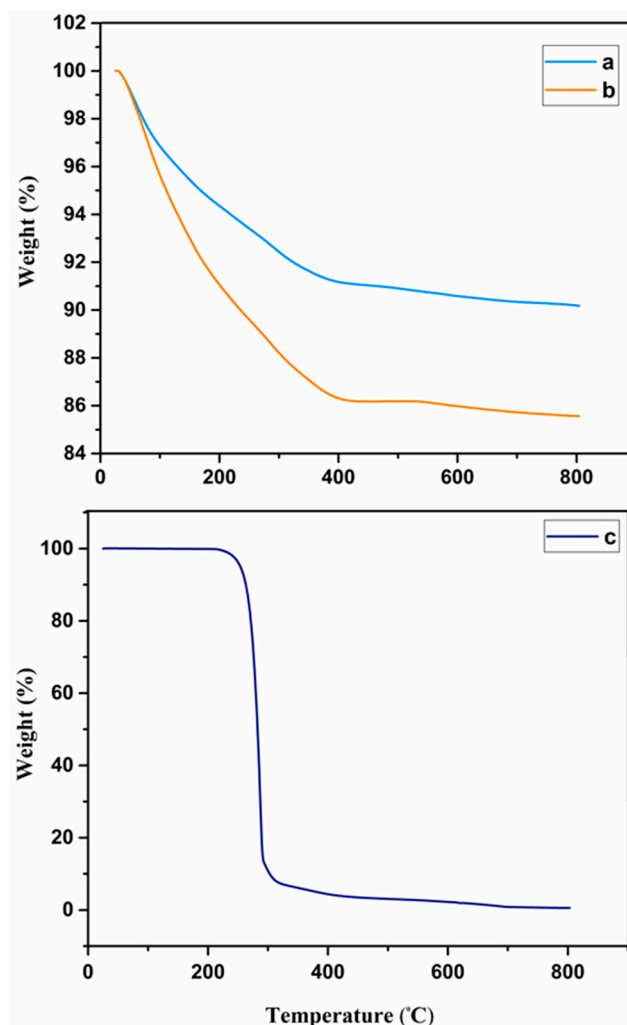


Figure 4. Thermogravimetric curves of NiFe₂O₄ nanoparticles (a), Met-NiFe₂O₄ nanoparticles (b), and methionine (c).

3.2. Size Distribution and Zeta Potential Measurement

The average hydrodynamic size, particle size distribution, and average zeta potential of all nanoparticles were measured at the same concentration and pH values. The particle hydrodynamic size distribution graph was plotted using Gaussian theorem for NiFe₂O₄, Met-NiFe₂O₄, and Tet-Met-NiFe₂O₄ nanoparticles (Figure 5). All the calculated values are given in a tabulated form (Table 1).

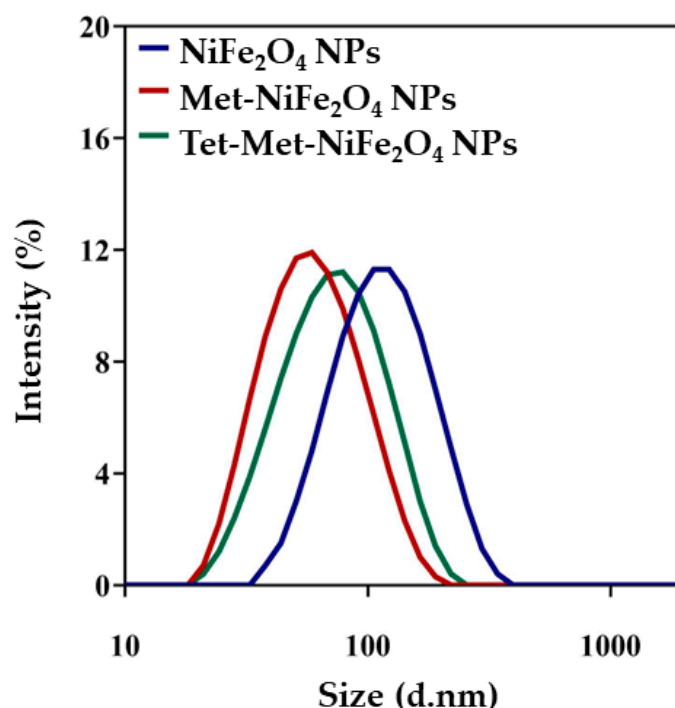


Figure 5. The particle hydrodynamic size distribution curves of NiFe₂O₄, Met- NiFe₂O₄, and Tet-Met-NiFe₂O₄ nanoparticles.

Table 1. Average hydrodynamic Size, PDI, and average zeta potential values of the fabricated formulations.

Formulation	Size (d.nm)	PDI	Zeta Potential (mV)
NiFe ₂ O ₄	168.5 ± 11.8	0.346 ± 0.02	−32.4 ± 2.4
Met-NiFe ₂ O ₄	72.4 ± 8.8	0.247 ± 0.06	−27.7 ± 4.3
Tet-Met-NiFe ₂ O ₄	90.9 ± 10.6	0.323 ± 0.02	−31.6 ± 1.2

3.3. Drug Loading and Release Study

Tet was further loaded onto Met-NiFe₂O₄ nanoparticles, and the drug loading and release profile was assessed. As shown in Figure 6A, the maximum loading capacity of Met-NiFe₂O₄ nanoparticles reached 0.055 mg/mg, when the initial Tet concentration was 0.09 mg/mL. In other words, 0.27 mg of Tet was loaded onto 1 mg of nanocarrier. The amount of drug loading is enhanced through increasing the initial drug concentration taken for the study. This is possible due to the larger surface area and hydrogen bonding between Tet and Met-NiFe₂O₄ nanoparticles. To evaluate the drug release profile, the Tet-Met-NiFe₂O₄ nanoparticles were suspended in the buffer media of pH 5 and 7.4. Figure 6B shows the pH-dependent release of Tet from Met-NiFe₂O₄ nanoparticles. Drug release was observed at pH values near the tumor's environment (pH 5), while the lower drug release was detected at pH 7.4. The pH-sensitive drug release of the nanocarrier under neutral conditions (pH 7.4) could reduce the drug loss during blood transportation and lessen the side effects of anti-cancer drugs observed in normal cells. In the simulated environment of tumors (pH 5), this above-mentioned property of nanomaterial facilitates efficient anti-cancer drug release [41]. The pH-responsive nano-based delivery tools could lead to site-specific release of therapeutic cargos through cleaving pH-sensitive bonds across the pH gradient and augment an increase in toxicity under acidic conditions prevalent in the tumor regions [42,43].

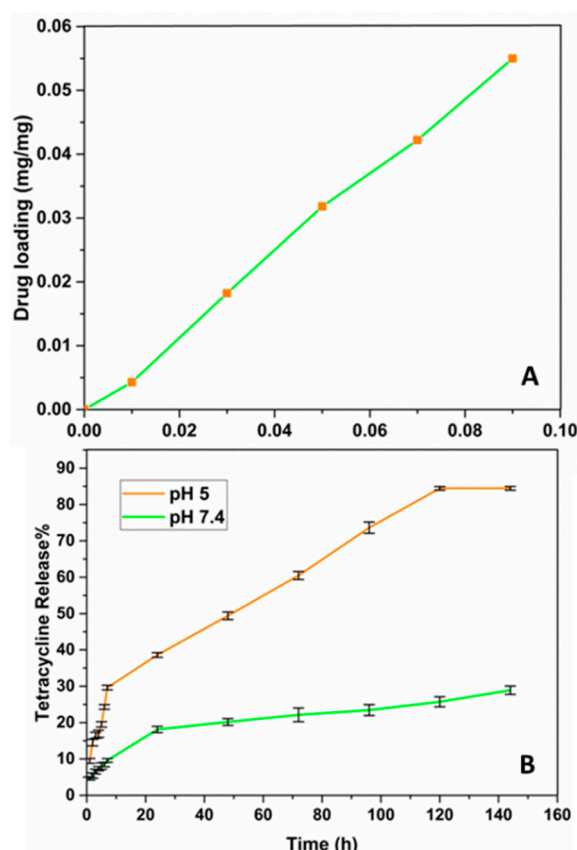


Figure 6. Tet loading on Met-NiFe₂O₄ nanoparticles (A) and Tet release from Met-NiFe₂O₄ nanoparticles at different pH values (B).

At neutral and acidic pH, the release kinetics of drug-loaded Met-NiFe₂O₄ nanoparticles were assessed using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, as shown in Table 2. An ideal kinetic model with a higher linear regression coefficient (close to 1) and determination (R^2) describe the drug release mechanism in the best way. The Higuchi model and Korsmeyer–Peppas model described the release kinetics at pH 5 and pH 7.4 ($n < 0.45$), whereas the Korsmeyer–Peppas model indicated the Fickian diffusion mechanism. It is worth mentioning that the drug gets distributed through diffusion in these cases (Higuchi model and Fickian diffusion mechanism). The pH-dependent drug release is well suited for cancer therapy, where the cancerous cells have an acidic intercellular environment while the healthy cells do not. The mathematical analysis is illustrated at pH 7.4 and pH 5 in Figures 7 and 8.

Table 2. The drug release kinetic models and the parameters obtained for optimum nanoformulation.

Kinetic Models	Equation	R^2	
		Met-NiFe ₂ O ₄ Nanoparticles (pH=7.4)	Met-NiFe ₂ O ₄ Nanoparticles (pH=5)
Zero-Order	$C_t = C_0 + K_0t$	0.8906	0.9516
First-Order	$\text{Log}C = \text{Log}C_0 + K_t/2.303$	0.9080	0.9809
Higuchi	$Q = K_h\sqrt{t}$	0.9712	0.9885
Korsmeyer-Peppas	$M_t/M_\infty = Kt^n$	0.9879 (* $n = 0.3775$)	0.9852 (* $n = 0.4261$)

* Diffusion or release exponent.

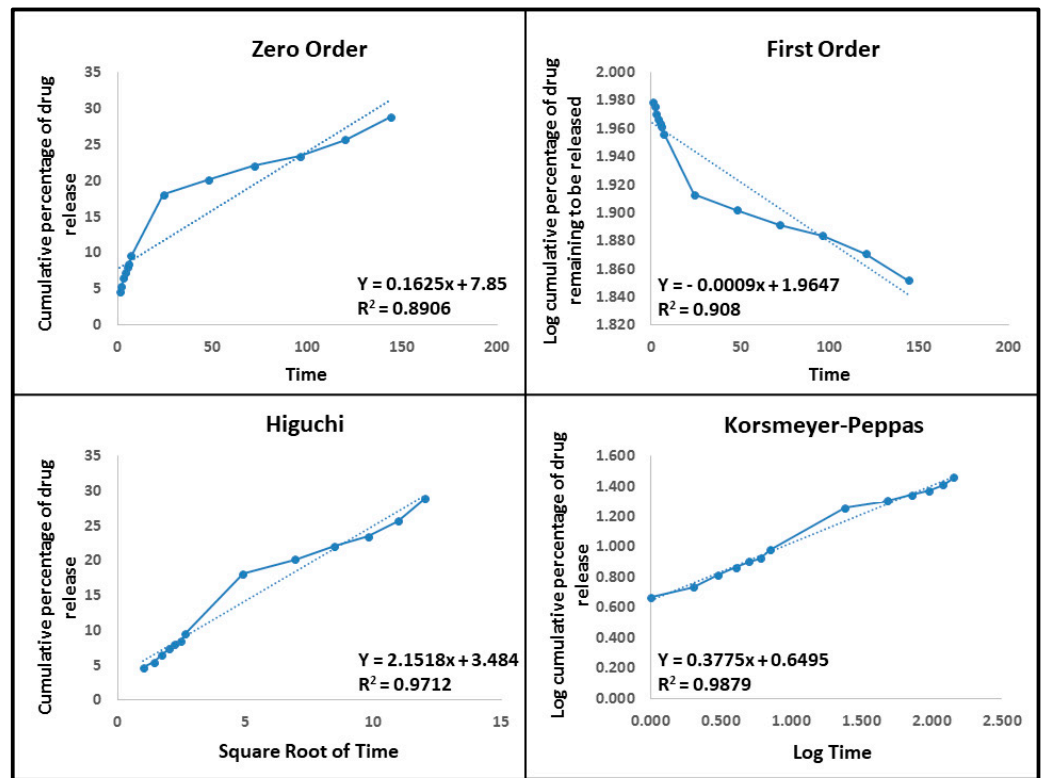


Figure 7. Drug release kinetic models like zero-order, first-order, Higuchi, and Korsmeyer–Peppas models used to study the tetracycline release from Tet-Met-NiFe₂O₄ nanoparticles at pH 7.4.

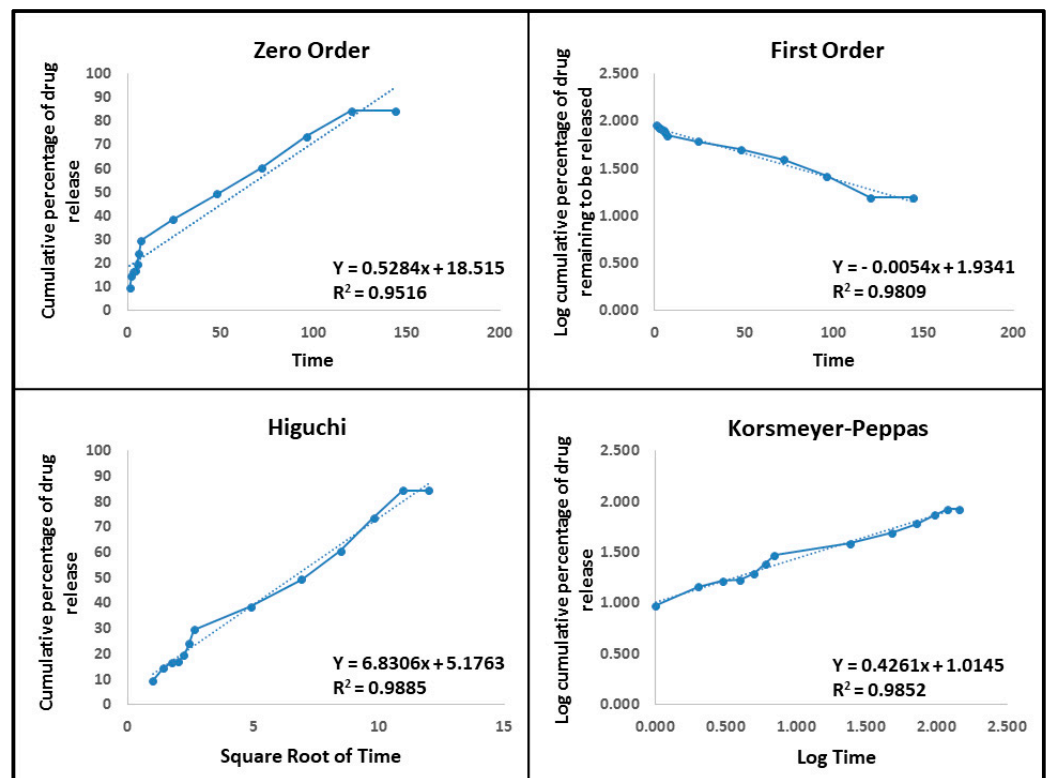


Figure 8. Drug release kinetic models like zero-order, first-order, Higuchi, and Korsmeyer–Peppas models used to study the tetracycline release from Tet-Met-NiFe₂O₄ nanoparticles at pH—5.

3.4. Cytotoxicity Studies

The cytotoxicity of Tet-Met-NiFe₂O₄ nanoparticles and free Tet were investigated using MTT test thrice for the concentration range of 0–70 µg/mL. Melanoma (A375 cells) and Human Foreskin Fibroblasts (HFF cells) were selected for the MTT assay. Results indicated that both free Tet and Tet-Met-NiFe₂O₄ nanoparticles showed cytotoxicity on cancer cells while toxicity was not observed with normal cells. As shown in Figure 9B, the cytotoxicity of Tet-Met-NiFe₂O₄ nanoparticles (IC₅₀ = 32.55 ± 7.31 µg/mL) was higher than free Tet (IC₅₀ = 76.58 ± 5.78 µg/mL) at 72 h. One possible reason for the differences in toxicity is due to difference in intracellular uptake of drug between the free drug and drug-loaded nanoparticles. More importantly, when the free drug is taken by cells, its release is faster, which means it is available to cell in less time owing to cross-sectional effect. When the drug is loaded onto nanoparticles, it exhibits controlled release, sustained effect, and as a result, the overall therapeutic efficacy is significantly improved [22,44]. Furthermore, Tet-Met-NiFe₂O₄ nanoparticles displayed no cytotoxicity on HFF cells (Figure 9A) due to excellent biocompatibility observed through coating the MNPs with amino acid methionine. These results finally showed that the biocompatible Tet-Met-NiFe₂O₄ nanoparticles synergistically improved the growth inhibitory effect on cancer cells.

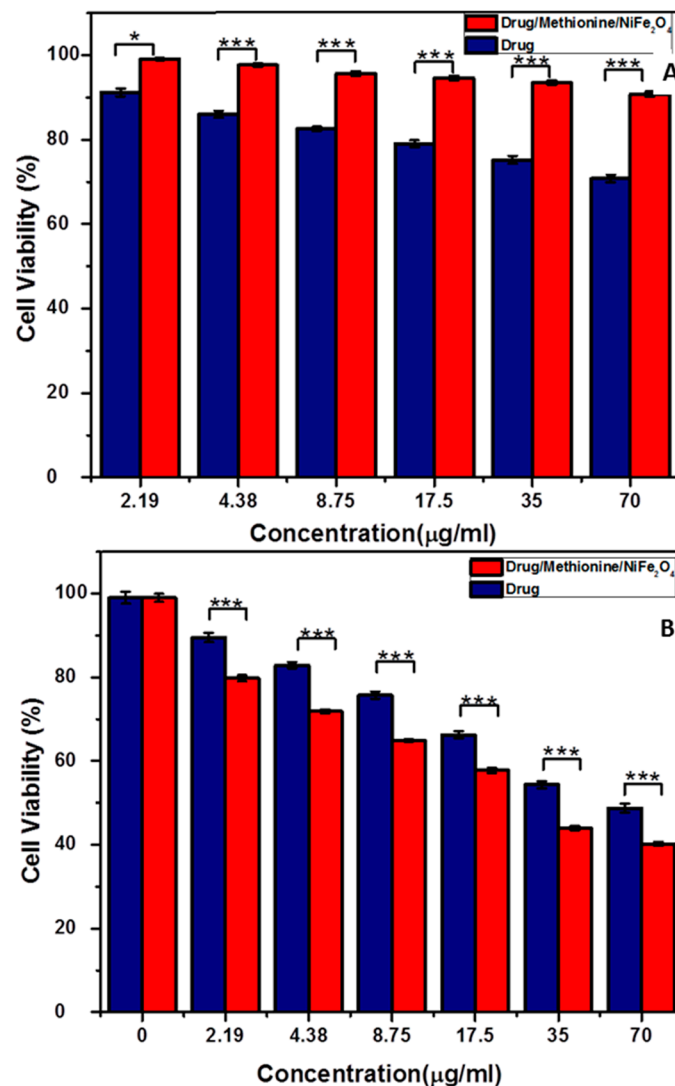


Figure 9. Cell viability of Tet-Met-NiFe₂O₄ nanoparticles and free Tet solution after 72 h incubation with (A) HFF cells and (B) A375 cells. The data is expressed as mean ± SD (*n* = 5). * *p* < 0.05, *** *p* < 0.001.

3.5. Antibacterial Activity

Microdilution method was used to evaluate the antibacterial activity of free Tet and Tet-Met-NiFe₂O₄ nanoparticles. Table 3 showed that MIC of Tet-Met-NiFe₂O₄ nanoparticles against pathogenic bacteria were between 1 to 8 µg/mL. However, Tet-Met-NiFe₂O₄ nanoparticles had potent antibacterial activity against Gram-negative bacteria with a significant reduction in MIC and MBC values i.e., 2–3-folds as compared to that of free Tet (Table 3). This was probably due to more penetration of Tet-Met-NiFe₂O₄ nanoparticles into Gram-negative bacterial cells [45]. In fact, Gram-negative bacteria possess very thin peptidoglycan layer than Gram-positive bacteria, and this structure may facilitate the penetration of nanoparticles into bacterial cells [46–49]. These data supported the idea that Met-NiFe₂O₄ nanoparticles can be utilized as carriers of antibiotics for antibacterial applications. Another probable antibacterial mechanism of Met-NiFe₂O₄ nanoparticles could be due to enhanced bacterial outer membrane permeability, which is due to the interaction of nanoparticles with the bacterial cell walls, which, in turn, alters the intrinsic membrane potential [50].

Table 3. Antibacterial activity of free Tet and Tet-Met-NiFe₂O₄ nanoparticles.

Bacteria		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. faecalis</i>
MIC (µg/mL)	Free Tet	8	8	8	8
	Tet-met-NiFe ₂ O ₄	1	2	4	4
MBC (µg/mL)	Free Tet	16	16	16	16
	Tet-met-NiFe ₂ O ₄	2	2	8	8

4. Conclusions

MNPs have been broadly explored for the development of drug delivery systems in recent years. In this study, Tet-Met-NiFe₂O₄ nanoparticles was fabricated for cancer therapeutics. Results suggested that methionine coating onto magnetic NiFe₂O₄ nanoparticles stabilize the nanoparticles and, thus, lead to more effective drug loading. Evidently, Tet was released rapidly into the cancer cells. In vitro cytotoxicity test indicated the excellent biocompatibility of magnetic nanocarriers due to the surface coating with methionine. When Met-NiFe₂O₄ nanoparticles were tested, no cytotoxicity was observed on both cell lines. Tet-Met-NiFe₂O₄ nanoparticles showed significant cytotoxicity on A375 cells, even higher than free Tet solution.

Furthermore, strong bactericidal activity was seen, when Tet-Met-NiFe₂O₄ nanoparticles was tested against different Gram-positive and Gram-negative bacterial pathogens. Collectively, these results presented that Met-NiFe₂O₄ nanoparticles like biocompatible nanocarriers could be potentially utilized for treating bacterial infections and cancer.

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