Cefotaxime removal enhancement via bio-nanophotocatalyst α-Fe$_2$O$_3$ using photocatalytic degradation technique and its echo-biomedical applications

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The present paper evaluates the photocatalytic degradation (PCD) performance of the biofabricated hematite nanoparticles (α-HNPs) for the degradation approach of the Cefotaxime (Cfm). The optimum pH of the solution to achieve the best PCD was found to be 10.5. The kinetics study for the PCD of the Cfm via α-HNPs has been investigated and the reaction was found to be follow pseudo-first-order at $R^2 = 0.992$. The mass loading impact of α-HNPs was investigated and estimated for the maximum degradation of Cfm $0.4$ mg/mL. UV–Vis confirmed that α-HNPs had a direct transition bandgap at $3.78$ eV at a maximum absorption wavelength of $362$ nm with suspension stability for 7 days. The probable mechanism of the Cfm PCD via α-HNPs and the degradation pathway was conducted. The validation of the suspension stability of the α-HNPs ($-68.6 \pm 11.8$ mV) was determined using the zeta potential investigation test. XRD investigation was conducted after Cfm PCD showing an average crystallite size of $27.0$ nm. XRD, TEM, SEM, EDX, and FT-IR analyses have been conducted for the α-HNPs before and after Cfm PCD confirming the high efficiency for the reusability of the current biocatalyst α-HNPs for further use. TEM results of the particle sizes of α-HNPs were found at $19.2 \pm 4.4$ and $20.6 \pm 7.4$ nm respectively before and after Cfm PCD. The efficiency of the Cfm PCD was found to be $99.1\%$ after 6 h. High potent as an antibacterial agent of α-HNPs was investigated either α-HNPs alone or after its PCD activity against Cfm. The antibacterial activity revealed high sensitivity, especially toward Gram-positive species indicating its promising ability against pathogenic issues. Interestingly, Cfm@α-HNPs showed superior anti-proliferative activity as tested by MTT assay and were able to induce apoptosis in MCF7 and HepG2 cell lines using the flow cytometry technique at $20.7\%$ and $17\%$ respectively. Also, The IC$_{50}$ of hydrogen peroxide scavenging was estimated and it was manifested that $635.8$ and $665.6$ μg/mL of α-HNPs before and after the PCD process of Cfm respectively.

The necessity to treat wastewater is a critical issue in the world especially. There are several ways for water to become polluted. Contamination from dyes, pharmaceuticals, and industrial waste is one of these sources$^1$. Water containing organic pollutants such as industrial pharmaceutical waste and chemicals generated by textile industries has an impact on the biological cycle, specifically the photosynthesis process in plants, which has a direct impact on marine species and an indirect impact on human life$^2$. Furthermore, polluted water has a significant influence on the life of birds, animals, and humans who are impacted by contaminated flowing water$^3$.

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Pharmaceuticals have been broadly spectrum used in the last four decades in human, sustainable agriculture, aquaculture, and vet application areas for bacterial infection treatment especially for enhancing the quality of human life. Pharmaceuticals are an emerging environmental hazard due to the extremely large use of antibiotics that can easily be found in the aqueous environment, especially those that are classified as water-soluble drug substances. These drug constituents have been observed in drinking water, surface water, groundwater, and sewage effluent. The pharmaceutical drug substances can reach the aquatic environment via diversified sources such as pharmaceutical industrial wastewater, Hospital drains, and plants for conventional wastewater treatment. Of the diverse pharmaceutical drug products, antibiotics have been of considerable environmental concern due to the emergence of antibiotic-resistant bacteria which couldn't be treated with currently recognized prescription, and the resulting rise in their chemical toxicity. Cefotaxime (Cfm) is a typical example of an antibiotic. It is classified from the cephalosporin family of beta-lactam as one of their third-generation member. It has an antibiotic broad-spectrum activity as an antibacterial agent against either gram-positive or gram-negative micro-organism species.

Many studies reported that the Cfm in the effluents after the wastewater treatment of the plants, hospitals, drain, and sewage treatment facilities could be detected at a concentration greater than 300 mg/L. Although the toxicity of Cfm concentration in the aquatic environment is relatively low, the augmentation of resistant bacteria and resistance genes as a result of Cfm use is a more immediate issue. Furthermore, because of its biodegradability, Cfm cannot be successfully removed by usual wastewater treatment techniques. As a result, innovative alternative solutions to the antibiotic contamination problem are necessary. León et al. reported a removal efficiency of 84.2% of 20 mg/L of Cfm removal photocatalytic method via 2.5 g/L of TiO2. Also, in a recent study mesoporous C3N4 was used as a photocatalyst by Mengmeng et al. 22 in 1 g/L to remove the Cfm residual in a concentration of 2 mg/L in 60 min with an efficiency of 99%.

Several treatment approaches have been introduced to overcome this environmental issue, such as conventional methods such as biosorption, coagulation, membrane process, sedimentation, flocculation, filtration, and adsorption. Owing to the unsatisfactory efficacy and often lack of utility of these methods, modern alternative advanced oxidation processes have arisen. The later methods included different processes of chemical oxidation (O2, H2O2), photochemical (UV/H2O2), and photocatalysis (TiO2/ZnO/UV) that were used to generate the radical oxygen species (ROS) as hydroxyl radical (·OH). ROS has high activity as an oxidizing agent against various characterization techniques. As a result, the separation and recycling of ultrafine catalysts from treated wastewater are problematic, which could also pollute the treated water and is time-consuming and costly. These issues can be overcome by immobilizing the solid support PCD, which has an outstanding capacity to isolate Nano-sized materials and is cost-effective. There is considerable interest in the wastewater treatment approach development that inevitably involves photocatalysts based on semiconductor nanomaterials especially transition metal oxides like BiVO4, WO3, ZrO2, TiO2, MnO2, Fe2O3, CuO, Cu/TiO2, ZnO, ZnO/Sb, MoO3, and Cu/Cu2O/Cu nanocomposite. Among all of these, Alpha Hematite Nanoparticles Fe2O3 (α-HNPs) offers several advantages such as low-cost, chemical stability, nontoxicity, and wide band gap. To the best of our knowledge, we conduct herein for the first time photocatalytic of Cfm using α-HNPs. In this work, nanostructured α-HNPs (28.01 g/m2) were prepared as previously reported using a greener approach. The resulting α-HNPs were characterized using various characterization techniques. The objectives of this investigation are to gain a mechanistic pathway in PCD of the Cfm wastewater-contaminant as an antibiotic model.

Materials and methods

Chemicals and reagents. All chemicals used in the current work were analytical grade. Methanol HPLC grade from (Merck). Cfm standard (C16H17N5O7S2); molar mass (455.46 g/mole); purity (96.3%) Zhuhai United Laboratories Co. Ltd (India) was kindly supplied by UP pharma (Assiut, Egypt). Disodium hydrogen phosphate and Hydrochloric acid, Phosphoric acid 85%, Sodium hydroxide, and Hydrogen peroxide (Scharlau, Spain). Deionized water used in the analysis was prepared by reverse osmosis and passed through a 0.45 μm Millipore filter (Millipore Company, USA) before use.

Instrumentation. UV–vis absorption measurement of the α-HNPs absorption was recorded in the range 200–800 nm using PerkinElmer [LAMBD A 40] Spectrophotometer using a quartz cell of 1 cm path length at
room temperature. Malvern Zeta Sizer equipped with a 4mW helium/neon laser wavelength equals 633 nm at 25 °C (Nano-ZS). The XRD parameters as crystal lattice size “D” could be determined using the Scherrer equation for each peak. Also, the other XRD parameters could be estimated as the strain of the lattice (E), placing distance (d), dislocation density (δ) and stacking fault (α), crystallinity (%), and crystallinity index (CI) as manifested in the supplementary material file; Eqs. (1–7). FT-IR analysis was recorded on a Thermo Fisher [Nicolet i510 FT-IR spectrometer] in a wavenumber range of 4000–400 cm⁻¹ using an ATR module. The particle size of the α-HNPs before and after Cfm PCD was conducted using transmission electron microscopy [TEM; JEOL JEM-100C XII]. The morphology and chemical composition of the α-HNPs and Cfm@α-HNPs were investigated using scanning electron microscopy [SEM; JSM IT 200]. Cfm quantitative analysis and its related substances were determined using the LC-20A HPLC instrument with the PDA (Shimadzu). The method was performed on the (Thermo Scientific) RP column BDS (150 mm × 4.0 mm × 5 μm) with a PDA detector at 235 nm, column oven at 30 °C, and injection volume of 20 μL. Flow cytometry was carried out using FACS-Calibur flow cytometer (Becton Dickinson, USA) and data were analyzed using FlowJo software (Treestar, Ashland, OR).

Methods and experiments. The photocatalytic efficiency studies were conducted under direct sunlight irradiation from 11.00 AM to 5.00 PM. All the study experiments were implemented according to the previously reported Cfm HPLC analysis method by Al-Hakkani. As a general procedure, after any process of Cfm solution and α-HNPs treatments the samples were filtered using nylon filter paper with a pore size of 0.45 μm using the Buckner filtration system. Then as an assurance procedure, an additional filtration step was performed using a syringe filter of 0.2 μm before introduction to the HPLC for analysis. To identify the related substances that were produced from the Cfm degradation; individual impurities (A, B, C, E, and F) were injected under the same analysis circumstances according to European Pharmacopoeia. The resolution between the nearest adjacent peak and the Cfm principle peak should be more than 1.5 according to the guidelines of the analytical method validation protocols.

Batch mode experiments. The placebo samples “Cfm only without any processes applied as adsorption, photolysis or PCDs” were conducted as a zero time for each experiment individually for each parameter for the whole of the study. Also, samples after 2 h of adsorption in the dark under continuous stirring at 350 rpm were conducted before starting the photocatalytic study to exclude the adsorption/desorption effect.

The degradation % was calculated using the equation in the supplementary material file; Eq. (8).

Interactive effect of solution pH change. Lopez-Alvarez et al. reported that the most important parameters affecting the PCD process are the pH of the solution and catalyst dose. So, both of them and the time effect parameter were to be evaluated.

The photolysis and photocatalytic studies were conducted for 6 h via direct sunlight for 250 mL of Cfm 20 mg/L using 250 mg of as-biofabricated α-HNPs with continuous stirring at 350 rpm. The impact of solution pH on the Cfm degradation was implemented firstly by maintaining all the parameters fixed at different pH medium solutions in the range (2.5–12.5). Hydrochloric acid 0.1 M and sodium hydroxide 0.1 M were used for the pH adjustment. The pH of the solution was adjusted before starting the sunlight irradiation and is not controlled while conducting the degradation reaction.

Kinetic mechanism studies. To determine the time effect “kinetic study profile”, 250 mL of Cfm 20 mg/L at pH 10.5 samples were prepared in a beaker containing 250 mg of the as-biofabricated α-HNPs under continuous stirring at 350 rpm. At different time intervals (1, 2, 3, 4, 5, and 6 h); the degradation % of Cfm was estimated.

The rate constants of the Cfm PCD can be estimated via the pseudo-first-order and second-order equations as in the supplementary material file; Eqs. (9,10).

Influence of the as-biofabricated α-HNPs catalyst loading. For the investigation study of the as-biofabricated α-HNPs mass on the photolysis and photocatalytic activities of Cfm, 250 mL of Cfm solution 20 mg/L at pH 10.5 samples were prepared. Solutions were taken individually in a beaker at different quantities of α-HNPs at (0.04–1.0 g/L). Then the samples were exposed to direct sunlight with continuous stirring at 350 rpm.

Practical application using an actual pharmaceutical wastewater sample after production of Cfm. Initially and before the photocatalytic process application, the physicochemical parameters as solution pH, conductivity, total dissolved solids (TDS), and zero-time HPLC assay were analyzed. In a 100 mL beaker; 50 mL of an actual pharmaceutical wastewater sample that was previously filtered through a nylon membrane filter of 0.45 μm, the α-HNPs were added to get a concentration of 1.0 mg/mL with continuous stirring for 2 h at 350 rpm at room temperature. Consequently, the sample was left in direct sunlight from 11:00 AM to 04:00 PM. After completion of the reaction, the sample was filtered using nylon filter paper with a pore size of 0.45 μm using the Buckner filtration system, then an additional filtration step was performed using a syringe filter of 0.2 μm before introduction to the HPLC instrument for analysis.

Antibacterial activity. Four examples were used for the investigation of the antibacterial activity of the as-prepared α-HNPs against Cfm@α-HNPs after Cfm degradation. Gram-negative bacteria species were tested as Escherichia coli (E. coli) and Salmonella typhimurium (S. typhimurium). While Enterococcus faecalis (E. faecalis) and Staphylococcus albus (S. albus) were tested as examples of Gram-positive bacteria. The bacterial types were kindly provided by the lab of Microbiology; Faculty of Science; New Valley University, Al-kharga, Egypt. The
antibacterial activity was investigated via the agar well diffusion according to Magaldi et al.\textsuperscript{1,46}. In brief, the wells were made by punching the nutrient agar surface with the sterile cork borer (8 mm diameter). The Dimethyl sulfoxide (DMSO) was used as a negative control where all testes were suspended in DMSO as a solvent. Cefotaxime sodium (Cephalosporin third-generation antibiotic) 250 µg/mL was used as a positive control. \textit{Echinacea purpurea} (\textit{E. purpurea}) and the as-prepared \textit{α-HNPs} against \textit{Cfm@α-HNPs} after \textit{Cfm} degradation were tested at a concentration of 1000 µg/mL. All tests were added to the wells. The Petri dishes were incubated at 30°C for 24 h, and the clear inhibition zones were estimated in mm and recorded.

**Anti-proliferative activity.** MTT assay was used to evaluate the effect of the different compounds on the proliferation of MCF7 cells (breast carcinoma cell line) and HepG2 cells (Human liver Hepatoma carcinoma cell line). About 5 x 10^4 cells were inoculated in wells of 96 well tissue culture plates. Then cells were treated with serial dilutions of the tested samples and incubated at 37°C overnight. MTT solution (Promega, USA) was applied at the recommended concentration to cells for 3 h and the formed formazan crystals were solubilized. The optical density was measured at 560 nm and the anti-proliferative activity of \textit{E. purpurea} liquid extract, \textit{α-HNPs}, and \textit{Cfm@α-HNPs} were evaluated against control untreated cells as previously described.

To confirm these results, MCF-7 cells were treated with the \textit{E. purpurea} liquid extract, \textit{α-HNPs} and \textit{Cfm@α-HNPs} at a concentration of 100 µg/ml for 24 h, and apoptotic cells were evaluated using flow cytometry. Following incubation with the indicated test samples, cells were stained with Annexin V-FITC and propidium iodide (FITC Annexin V Apoptosis Detection Kit with PI, Biolegend, USA) to evaluate the frequency of late apoptotic cells which acquired both dyes.

**Hydrogen peroxide scavenging (H$_2$O$_2$) assay.** The ability of as-biofabricated \textit{α-HNPs} to scavenge hydrogen peroxide can be determined according to the reported method by Ruch et al.\textsuperscript{47}. A solution of 0.004 M of hydrogen peroxide was prepared using the 0.05 M phosphate buffer pH 7.4 solution as a solvent. The blank solution was conducted using a phosphate buffer solution in absence of the hydrogen peroxide. The standard hydrogen peroxide concentration was estimated spectrophotometrically via absorption measurement at 230 nm as ($A_b$). The as-biofabricated \textit{α-HNPs} were suspended in the distilled water subsequently, they were added to the hydrogen peroxide/phosphate buffer in a final concentration range (10–1000 µg/mL). Finally, the absorbance at 230 nm was determined after 10 min as ($A_t$).

The scavenging percentage of hydrogen peroxide could be calculated as in the supplementary material file; Eq. (11).

**Results and discussion**

The retention time (Rt) of the \textit{Cfm} peak was found to be about 12.5 min. Also, the Rt of \textit{Cfm}-related substances was determined and found to be at about 2.9, 4.4, 8.2, 9.6, and 14.6 min for impurities B, C, E, A, and F respectively.

The \textit{Cfm} principle peak was found to be resolved from the impurity F at an excellent value via 8.11 as revealed in Fig. 2. Any other impurity less than the reporting level at 0.05% of the \textit{Cfm} peak area in the chromatogram will be disregarded according to the European Pharmacopoeia guideline\textsuperscript{49}.

**Interactive effect of solution pH change.** The pH of the solution in diverse photocatalytic systems is a factor that can affect the polarization of the catalyst and the dissociation of the substrate, which can determine the various electrostatic interactions among the present species in the reaction medium\textsuperscript{1,46,49}. 

![Figure 2. Cefotaxime and its related substances HPLC chromatogram.](image-url)
To study the solution pH effect on the degradation of Cfm; some experiments were implemented in solution pH within the range of 2.5–12.5 maintaining the rest of the experiment factors fixed at 1.0 g/L of α-HNPs loading dose and 250 mL of 20 mg/L of Cfm concentration for 6 h.

According to Fig. 3a; the comparison profiles for both photolysis and photocatalytic for Cfm degradation; it can be concluded that there is no effect of the sunlight alone on the Cfm degradation over at the full pH range 2.5–12.5. On the other hand, the photocatalytic of the Cfm degradation increased dramatically (43.9–90.6%) in the pH range 2.5–8.5 showing the maximum degradation of Cfm (99.8%) at pH 10.5 followed by decreasing at pH 12.5 (68.5%).

The Cfm degradation usually depends on the adsorption of its molecules onto the α-HNPs surface and the generation of the hydroxyl free-radical which depends on the availability of hydroxyl ions in the reaction medium. As a result of the previous determination of the zero-point charge (pHzpc) of α-HNPs that was found to be 5.2. So, at the pH of solutions below this value; the α-HNPs surface will be positively charged. On the contrary, at pH solutions more than pH 5.2, the α-HNPs surface will be negatively charged. Cfm shows three pKₐ (acid dissociation constant) values at 2.1, 3.4, and 10.9 as León et al. reported. So, the Cfm at pH range (4–10.5) presents as negatively charged ions.

According to the attraction electrostatic force among the α-HNPs surface charges and the Cfm ions under the dedicated pH reaction medium (2.5–4.5), the promotion to generate the hydroxyl radicals was enhanced.
The increase in Cfm degradation efficiency % at the pH range (6.5–10.5) may be attributed to hydrogen bonding formation between the Cfm and the adsorbed bioactive molecules that surrounded the as-biofabricated α-HNPs or π–π interaction\(^1\)\(^{26,37}\) as manifested in Fig. 3b. Completely, Cfm dissociation at pH 12.5 or more causes the presence of the Cfm species as deprotonated anions and the negatively charged surface of the as-biofabricated α-HNPs maybe it is the main cause of the repulsion electrostatic force. That was subsequently, followed by a decrease in Cfm degradation.

**Kinetic mechanism studies.** The Cfm responses at zero time, after adsorption, and after the PCD at different time intervals (1–6 h) were manifested in Fig. 4a. It is very clear that the efficacy of the as-biofabricated α-HNPs on Cfm degradation as a photocatalytic agent. The Cfm peak response was decreased rapidly as a function of degradation time.

The first rapid PCD was observed at the first hour of the reaction and it was found to be 47.2%. The increase of PCD efficiency almost reached the maximum after 4 h at (96.4%). After that, the progress in the degradation was limited where it was increased up to 97.9% followed by a slight increment at 99.1% after further 2 h of sunlight time exposure as depicted in Fig. 4b. So, the critical photocatalytic time interval is represented after 4 h of the reaction.

As the HPLC degradation profile manifested (Fig. 4c and Table 1); the Cfm further degradation resulted from related substances (impurities A, B, C, E, and F) showed a notable increase in their responses. The most abounded of these related degradant substances was impurity C which gave an increased assay response factor at a ratio from <0.05% at Cfm zero-time assay to 1.36% at the end of catalytic degradation after 6 h. Impurity A came in the second-order of the increase in its assay response by 1.32% from the Cfm zero-time assay. Impurities E, B, and F came posteriorly in degradation ratios assay as 1.00%, 0.27%, and 0.14% respectively.

It is clear that the total impurities assay was about 3.82% of the principle Cfm peak, this ratio reflects the strength of the as-biofabricated α-HNPs toward Cfm degradation for its primary components as carbon dioxide and water.

Figure 4d shows that the Cfm degradation fits appropriately with first-order kinetic according to the \(R^2\) value which was found to be 0.992. The rate constant of the Cfm was found to be 0.8325 h\(^{-1}\).

**Influence of the as-biofabricated α-HNPs catalyst loading.** It is clear to man that without using the α-HNPs catalyst (photolysis) there was no significant degradation change over the reaction time of 6 h and it can be neglected as shown in solution pH and time change effect.

For studying the effect of the as-biofabricated α-HNPs loading, a series of experiments were conducted for 4 h at different doses of the α-HNPs in the range 0.04 to 1.0 g/L maintaining the Cfm concentration at 20 mg/mL and pH of 10.5 as shown in Fig. 5.

The photocatalytic is a surface phenomenon so, it strongly depends on the crystallinity nature and the surface area \(28.01 \text{ g/m}^2\) as previously reported in our approach\(^{50}\) that is proportional directly to the mass loading of the catalyst under study. According to the increase of the as-biofabricated α-HNPs loading, along with reaction time, there was a drastic rise in the degradation rate (58.5%, 83.4%, 96.6%, and 99.6%). So, it was found to be the degradation profile is properly dose-based. The increment efficiency of degradation may be attributed to the photon absorption via the as-prepared α-HNPs that cause the generation of the hole-electron pairs\(^1\). So, the total activated surface area increased with increasing the catalyst dose which subsequently, increase the reactive oxygen species production as revealed in the photocatalytic degradation mechanism scheme equations. Any increase in the α-HNPs after 0.4 g/L loadings had no notable effect on the increase of Cfm degradation efficiency compared with the loading dose effect at (0.04–0.2 g/L). So, it can be negligible because it was an almost slight increase. This behavior may be due to the agglomeration of NPs, solution opacity, or scattering of the light at higher doses of the as-prepared α-HNPs\(^{51}\). So, 0.4 g/L can be selected as a convenient dose for further studies.

**Photocatalytic activity comparison studies of the biosynthesized α-HNPs.** From the last decade and up to date, the biofabricated α-HNPs via various parts of different kinds of plants were paid attention to and gained great interest in several applications, especially in photocatalytic activity against some of the organic substances as reported in Table 2.

**UV–Vis analysis.** The optical characterizations of the biosynthesized α-HNPs powder sample were investigated after it was dispersed in deionized water. The α-HNPs exhibited the maximum absorption peak in the UV range at 362 nm Fig. 6a, this result was found to be compatible with the previously reported surface plasmon (SPR) peaks by Rajiv et al.\(^{60}\) and Al-Hakkani et al.\(^{66}\). The stability of the α-HNPs suspension form in the deionized water was tested for one week by examining for the distinct SPR peak in the optical spectrum and it was found to be at the same wavelength. The bandgap energy of the as-prepared α-HNPs could be determined using the optical as manifested in the supplementary material file; Eqs. (12, 13) \(^1\). The \(E_g\) of the direct transition was found to be at the same wavelength. The bandgap energy of the α-HNPs could be determined using the optical as manifested in the supplementary material file; Eqs. (12, 13) \(^3\). The \(E_g\) of the direct transition was found to be at the same wavelength. The bandgap energy of the as-prepared α-HNPs might be attributed to the presence of the Cfm species as deprotonated anions and the negatively charged surface of the as-biofabricated α-HNPs maybe it is the main cause of the repulsion electrostatic force. That was subsequently, followed by a decrease in Cfm degradation.

**Zeta potential.** The suspension stability of the α-HNPs could be estimated using the value of the zeta potential instrument\(^8,36\). The value of zeta potential of α-HNPs suspension stability in water was found to be \(-68.6 \pm 11.8 \text{ mV}\) which indicated that NPs had an excellent stability Fig. 7.

Zeta potential result was found to be compatible with the UV–Vis. α-HNPs suspension stability. Several studies reported that the colloidal/suspended particles had good stability if their charged surface passed the critical value ± 30 mV. Table 3 shows the relationship between the zeta potential value of the colloidal/suspended material...
Figure 4. (a) Cefotaxime degradation (%) against different time intervals using $\alpha$-HNPs. (b) Cefotaxime degradation (%) profile as a function in time. (c) Cefotaxime photocatalytic degradation after six hours via $\alpha$-HNPs (HPLC-related substances profile). (d) Kinetic models I) Pseudo-first-order, II) Second-order.
### Table 1. HPLC Cfm related substances impurities profile data after six hours of PCD via α-HNPs.

| Impurity | IUPAC Name                                                                 | Chemical name                                                                                     | Molecular formula | Molar mass (g/mol) | Rr (min) | Degradation (%) |
|----------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|-------------------|-------------------|-----------|-----------------|
| A        | \((6R,7R)-7-((Z)-2-(2-aminothiazol-4-yl)-2-((methoxy-d3)imino)acetamido)-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid\) | Cefetamet                                                                                         | C₁₄H₁₅N₅O₅S₂     | 397.43            | 9.601     | 1.32            |
| B        | \((6R,7R)-7-((Z)-2-(2-aminothiazol-4-yl)-2-(methoxymino)acetyl)amino)-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid\) | Deacetyl Cefotaxime                                                                            | C₁₄H₁₅N₅O₆S₂     | 413.43            | 2.934     | 0.27            |
| C        | \((6R,7R)-3-\(N\)-(acetyloxy)methyl)-7-\(N\)-(\(2Z\)-2-(2-aminothiazol-4-yl)-2-(methoxymino)acetyl)amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid\) | N-Formylcefotaxime                                                                             | C₁₇H₁₇N₅O₈S₂     | 483.48            | 4.458     | 1.36            |
| D        | \((5aR,6R)-6-\(N\)-(\(2Z\)-2-(2-aminothiazol-4-yl)-2-(methoxymino)acetyl)amino)-5a,6-dihydro\(3H,7H\)-azeto[2,1-b]furo[3,4-d][1,3]thiazine-1,7(4H)-dione\) | Deacetylcefotaxime Lactone                                                                     | C₁₄H₁₃N₅O₅S₂     | 395.41            | 8.272     | 1.00            |
| E        | \((6R,7R)-3-\(N\)-(acetyloxy)imethyl)-7-\(N\)-(\(2Z\)-2-(2-aminothiazol-4-yl)-2-(methoxymino)acetyl)amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid\) | Cefotaxime Dimer                                                                               | C₁₉H₁₈N₁₀O₁₂S₄   | 850.88            | 14.649    | 0.14            |

**Figure 4.** (continued)

**Table 1.** HPLC Cfm related substances impurities profile data after six hours of PCD via α-HNPs.

**Figure 5.** Cefotaxime degradation (%) against adsorbent of α-HNPs mass effect.
and its corresponding relative stability. Highly positive or negative values onto the charged surface generate major repulsion forces, whilst repulsion between particles with the same electrical charge inhibits the particle agglomeration and hence it gives good dispersibility1–3,36,62,63.

**Degradation mechanism.** Sunlight irradiation-induced α-HNPs for production of the ROS especially hydroxyl radical and superoxide anion that is mainly responsible for Cfm degradation. The probable degradation mechanism can be illustrated in Fig. 8.

| Plant name/part | Substrate                          | References         |
|-----------------|------------------------------------|--------------------|
| *Amaranthus dubius* leaf | Methylene orange dye | 52                 |
| *Cyperus rotundus* L. | Congo red dye | 53                 |
| *Carcuma Tea leaves* | Methyl orange dye | 54                 |
| *Cynometra ramiflora fruit* | Methylene blue dye | 55                 |
| *Pomegranate (Punica granatum)* seeds | Blue 4 dye | 56                 |
| *Mandarin peels “Citrus reticulums”* | Basic Maxilon Blue GRL, acidic Neolan Blue 2G dyes, and 2,6-dichlorophenol | 57                 |
| *Cynometra ramiflora fruit* seeds | Rhodamine-B dye | 58                 |
| *Carica papaya leaf* | Remazol yellow RR dye | 59                 |
| *E. purpurea* leaf | Cefotaxime | Current study |

**Table 2.** Photocatalytic activity of the biosynthesized α-HNPs via various parts of different kinds of plants.

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**Figure 6.** Spectra of the as-biofabricated α-HNPs (a) UV–Vis excitation absorption, (b) Tauc plot of direct transition energy bandgap.

**Figure 7.** Zeta potential of the as-biofabricated α-HNPs.
According to the smallest energy band gap of the as-biofabricated semiconductor α-HNPs (3.78 eV) and also as a previously reported\(^3\), it can be easily excited via direct sunlight in the visible range and this is the main cause of the NPs photocatalytic activity as summarized in photocatalytic degradation mechanism scheme Eqs. (1–15).

Briefly, when most of the NPs were irradiated under the sunlight induction, the electrons were being promoted to the highest energy level leaving the valence band. The hole-electron pairs were being generated; holes occupy the valence band while electrons locate in the conduction band\(^3\).

Firstly, the holes (\(h^+\)) cause hydrolysis of water molecules to protons and hydroxyl ions which are subsequently, converted to hydroxyl radical. The ROS as hydroxyl radical considers the motive force in the oxidation of organic compounds where it acts as an oxidizing agent under convenient conditions. The final photocatalytic products are the primary components of any organic substances; that may undergo further degradation resulting water, carbon dioxide, and inorganic anions.

On the other hand, the negative charges (\(e^-\)) that are present in the conduction band induce the conversion of the adsorbed oxygen gas molecules to superoxide radicals which may directly degrade the organic substance or combined with the protons or holes forming an active hydroxyl radical species again and peroxides\(^5\). Finally, we can report that the various bioactive molecules present in the Echinacea extract which adsorbed at the NP’s surface, and the NPs themselves act as catalysts to promote photocatalytic activity.

The formation/elimination probabilities of the ROS during the PCD mechanism of Cfm via α-HNPs can be explored as the following mechanism scheme Eqs. (1–15):

![Diagram of Cefotaxime photocatalytic degradation probable mechanism via α-HNPs.]

**Table 3.** Zeta potential value stability indications.

| Zeta potential value (mV) | Colloidal/suspension stability indication |
|---------------------------|-----------------------------------------|
| −10, … , 0               | Flocculation or rapid coagulation        |
| −30, … , −10             | Relative (onset)                         |
| −40, … , −30             | Moderate                                 |
| −60, … , −40             | Good                                     |
| < −60                    | Excellent                                |
| 0, … , +10               |                                         |
| +10, … , +30             |                                         |
| +30, … , +40             |                                         |
| +40, … , +60             |                                         |
| > +60                    |                                         |

**Figure 8.** The Cefotaxime photocatalytic degradation probable mechanism via α-HNPs.
The Cfm PCD mechanism can be summarized as shown in scheme Eq. (15); firstly, an interaction of the Cfm with the photon energy forms an intermediate activated substrate. Subsequently, the latter can interact with any of the ROS final species as \( \text{O}_2/\text{O}_2^*/\text{OH} \) producing preoxylated or hydroxylated intermediate products that followed via degradation to the primarily related byproducts.

**Table 4.** Water physicochemical characteristics and assay of the Cfm.

| Characteristic parameter | Before | After |
|--------------------------|--------|-------|
| Concentration (mg/L)     | 9.7    | Not detected |
| Conductivity (μS/cm)     | 266.4  | 98.0 |
| TDS (mg/L)               | 131.0  | 44.2 |
| pH                       | 8.16   | 6.81  |

\[
[h\nu > E_g] + \alpha - \text{HNPs} \rightarrow e^- + h^+ \tag{2}
\]

\[
\text{H}_2\text{O} + h^+ \rightarrow \text{H}^+ + \cdot\text{OH} \quad (\text{Hydroxyl anion}) \tag{3}
\]

\[
\cdot\text{OH} + h^+ \rightarrow \cdot\text{OH} \quad (\text{Hydroxyl radical}) \tag{4}
\]

\[
\text{O}_2 + e^- \rightarrow \text{O}_2^- \quad (\text{Superoxide anion}) \tag{5}
\]

\[
\text{O}_2^- + e^- \rightarrow \text{O}_2^{2-} \quad (\text{Peroxide anion}) \tag{6}
\]

\[
\text{O}_2^{2-} + 2h^+ \rightarrow \text{H}_2\text{O}_2 \quad (\text{Hydrogen peroxide}) \tag{7}
\]

\[
\text{O}_2^- + \text{H}^+ \rightarrow \cdot\text{OOH} \quad (\text{Perhydroxyl radical}) \tag{8}
\]

\[
2[\cdot\text{OOH}] \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \tag{9}
\]

\[
\text{O}_2^- + e^- + \text{H}^+ \rightarrow \cdot\text{OH} \tag{10}
\]

\[
\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \cdot\text{OH} + \text{O}_2 \tag{11}
\]

\[
\text{H}_2\text{O}_2 + e^- \rightarrow \cdot\text{OH} + \cdot\text{OH} \tag{12}
\]

\[
\text{H}_2\text{O}_2 + e^- + \text{H}^+ \rightarrow \cdot\text{OH} + \text{H}_2\text{O} \tag{13}
\]

\[
\cdot\text{OH} + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O} \tag{14}
\]

---

**Practical application using an actual pharmaceutical wastewater sample after production of Cfm.** Water physicochemical characteristics and assay of the Cfm before and after photocatalytic process activity were evaluated and listed in Table 4.

The operating procedures revealed the efficiency of the photocatalytic activity in the industrial pharmaceutical wastewater treatment using \( \alpha\)-HNPs as a promising photocatalytic agent.

**XRD analysis.** The XRD diffractogram evidenced the highly crystalline form of \( \alpha\)-HNPs after Cfm PCD by the Bragg’s reflection peaks 2θ value and it corresponds to the lattice planes as manifested in Table 5. The dif-
fraction of the peaks reflected the presence of Trigonal (hexagonal axes) of hematite crystal with \( a = 5.0346 \, \text{Å} \); \( c = 13.7473 \, \text{Å} \) which is identical to the reference card ICDD No: 00-901-5964. These results are compatible with our earlier study Al-Hakkani et al.\(^3\) who reported the greener synthesis of \( \alpha \)-HNPs highly crystalline as sharpness peaks depicted in Fig. 9 with the evidence of lattice plans. Any other presence peaks may be attributed to the \( \text{Cfm} \) or their related substances from the adsorption process and/or \( \text{Cfm PCD} \) onto the surface of \( \alpha \)-HNPs\(^1,2\).

A slight increase of the average crystallite size (\( D \)) 27.0 nm after the \( \text{Cfm PCD} \) indicates the high efficacy of the reusability of the bio-nanocatalyst \( \alpha \)-HNPs for further use. Table 5 shows a comparison of the XRD parameters for the \( \alpha \)-HNPs before and after \( \text{Cfm PCD} \).

### Table 5. XRD parameters of the \( \alpha \)-HNPs after Cefotaxime photocatalytic degradation.

| Parameter          | Before          | After          |
|--------------------|-----------------|----------------|
| Crystallite size average (\( D \)) nm | 25.1            | 27.0           |
| Strain average (\( \varepsilon \)) \( 10^{-3} \) | 4.0 \( 10^{-3} \) | 3.3 \( 10^{-3} \) |
| Dislocation density average (\( \delta \)) lines/nm\(^2\) | 1.6 \( 10^{-3} \) | 1.6 \( 10^{-3} \) |
| Stacking faults average (\( \alpha \)) \( 10^{-3} \) | 2.5 \( 10^{-3} \) | 2.2 \( 10^{-3} \) |
| Crystallinity (%)  | 78.8%           | 78.7%          |
| Reference          | Al-Hakkani et al.\(^3\) | Current study |

Figure 9. XRD diffractogram of the \( \alpha \)-HNPs after Cefotaxime photocatalytic degradation.

### Table 6. XRD parameters comparison of the \( \alpha \)-HNPs before and after Cefotaxime photocatalytic degradation.

| Parameter | Before | After |
|-----------|--------|-------|
| Crystallite size average (\( D \)) nm | 25.1 | 27.0 |
| Strain average (\( \varepsilon \)) \( 10^{-3} \) | 4.0 \( 10^{-3} \) | 3.3 \( 10^{-3} \) |
| Dislocation density average (\( \delta \)) lines/nm\(^2\) | 1.6 \( 10^{-3} \) | 1.6 \( 10^{-3} \) |
| Stacking faults average (\( \alpha \)) \( 10^{-3} \) | 2.5 \( 10^{-3} \) | 2.2 \( 10^{-3} \) |
| Crystallinity (%) | 78.8% | 78.7% |
| Reference | Al-Hakkani et al.\(^3\) | Current study |
TEM analysis. The α-HNPs before and after Cfm PCD TEM analysis were being investigated as depicted in Fig. 10. Although there is an obvious very little increase in some of the particles which may be attributed to the adsorption process that was done before the degradation, but without any significant increment in particle sizes. Monodispersed cubic shapes were being manifested without any signs of particle agglomeration. The statistical calculations of the corresponding analysis were introduced in Table 7.

It’s clear to the man there is no change in the particle sizes of the α-HNPs before and after the Cfm photocatalytic process. This is an indication of the validity of α-HNPs for use to further run again without any significant change in the α-HNPs efficiency.

Morphological and chemical composition analysis (SEM and EDX). The surface morphology of α-HNPs before and after Cfm PCD was introduced as shown in Fig. 11. It’s clear and easy for man the distinguish the surface’s nature and changes that have been happened for each case of α-HNPs. EDX analysis showed a stronger proof...
to differentiate between $\alpha$-HNP$_n$ elemental composition alone and $\alpha$-HNP$_n$ after implementing the Cfm PCD process. EDX analysis in the case of $\alpha$-HNP$_n$ without any treatment process manifested that the main element components are only oxygen and iron atoms Fig. 11A. While after the Cfm PCD process that occurred at the surface of the $\alpha$-HNP$_n$ Fig. 11B we can see other elements that have appeared as carbon, nitrogen, sodium, and sulfur that are the main composition of Cfm and their degradation substances as it was manifested in Table 1. This result confirms the adsorption and degradation process of Cfm and its related substances at the $\alpha$-HNP$_n$ surface. Also, SEM analyses for $\alpha$-HNP$_n$ before and after the Cfm PCD process have been conducted showing an observable change as Fig. 11C revealed. Some of the particles are different in shapes that appeared as light-colored plates from the original dark-colored base of $\alpha$-HNP$_n$ Fig. 11D. These white plates could be attributed to the adsorption or degradation process of Cfm or their related substances at the $\alpha$-HNP$_n$ surface.

FT-IR analysis. FT-IR analysis plays an important role to affirm the functional groups’ encapsulation that adsorbed/adhered at the $\alpha$-HNP$_n$ surface Fig. 12$.^2$ There are some little functional groups dedicated to Cfm that have clear contributions from the adsorption process at the surface of $\alpha$-HNP$_n$ or some of the adsorbed degradant substances from Cfm molecules. This was exhibited especially in the fingerprint region of Cfm (2000–500 cm$^{-1}$) except in the Fe–O band (522 cm$^{-1}$)$^{1,36}$. This investigation assures the efficacy of $\alpha$-HNP$_n$ that could be used for multiple photocatalytic runs and this result was found to be compatible with the previously TEM findings.

Figure 11. $\alpha$-HNP$_n$ before and after Cefotaxime photocatalytic degradation EDX analysis (a, b); SEM analysis (c, d).
Antibacterial activity. Results showed that DMSO had no antibacterial effect where there are no clear zones appeared. On the contrary, the aqueous extract of \textit{E. purpurea} exhibited a moderate antibacterial activity almost was the same against both the Gram-positive and Gram-negative bacteria in the range of clear zone 12–14 mm. On the right-hand Cefotaxime manifested more activity against all tested bacterial species. The clear zones against \textit{E. coli}, \textit{S. albus}, and \textit{E. faecalis} were found to be 40, 38, and 38 mm respectively where it showed a clear zone of 20 mm against \textit{S. typhimurium}. The as-prepared Cfm@\textalpha-HNPs system exhibited an antibacterial activity better than \textalpha-HNPs alone against all tested bacterial types especially for Gram-positive bacteria more than Gram-negative bacteria. The measurements of the inhibition clear zones were depicted in Fig. 13. The difference in the clear zones in the antibacterial activity depends on the bacterial species’ ability. The results indicate that the Gram-positive bacteria are more sensitive to \textalpha-HNPs and Cfm@\textalpha-HNPs compared to Gram-negative bacteria. This could be explained by the high ability of the as-prepared NPs to penetrate the cell wall of Gram-positive bacteria. The
Table 8. Antibacterial activity of the biosynthesized α-HNPs via various parts of different kinds of plants.

| Plant name/part | Application                        | References |
|----------------|-----------------------------------|------------|
| Cynometra ramiflora | E. coli & S. epidermidis       | 58         |
| Carica papaya leaf  | Klebsiella spp., E. Coli, Pseudomonas spp. & S. aureus | 59         |
| Sida cordifolia               | B. subtilis, S. aureus, E. coli & K. pneumonia | 60         |
| Lantana camara leaf        | P. aeruginosa                     | 60         |
| Terminalia bellirica, Moringa oleifera fruit & Moringa oleifera leaves | S. aureus, B. subtilis & P. aeruginosa | 60         |
| Skimmia Laureola leaf       | R. solanacearum                   | 60         |
| Henna (Lawsonia inermis) leaf | S. aureus & S. typhimurium      | 61         |
| Rheum emodi root           | E. coli & S. aureus               | 61         |
| Laurus nobilis L            | L. monacynogenes                  | 61         |
| E. purpurea                 | E. coli, S. albus, E. faealis & S. typhimurium | Current study |

Table 9. Summary of IC\textsubscript{50} of the different tested samples on the proliferation rate of MCF7 and HepG2 cell lines.

| Tested product | Cell line IC\textsubscript{50} (µg/ml) | References |
|----------------|---------------------------------------|------------|
| E. purpurea    | MCF7 679.2 HepG2 643.4                | Al-Hakkani et al.\textsuperscript{36} |
| α-HNPs         | MCF7 141.6 HepG2 198.2                | Al-Hakkani et al.\textsuperscript{36} |
| Cfm@α-HNPs     | MCF7 105.31 HepG2 117.33              | Current study |

Figure 14. Cfm@α-HNPs have a marked anti-proliferative activity. MCF7 cells were treated with indicated samples and the frequency of late apoptotic cells was determined using flow cytometry.

The high activity of the Cfm@α-HNPs compared with α-HNPs alone may be attributed to the adsorption of some of the degradation substances after the Cfm PCD process that are deposited onto the α-HNPs surface and they have antibacterial activity. The following comparison in Table 8 showed different antibacterial activities of green biosynthesized α-HNPs using different plant extracts.

Anti-proliferative activity. Interestingly, in addition to the antibacterial activity, the biofabricated noncompounds exhibited enhanced antiproliferative activities. IC\textsubscript{50} against MCF7 cells decreased from 679.2 µg/ml when applying the plant extract to 105.31 µg/ml when Cfm@α-HNPs were used, pointing to marked anti-proliferative activity even compared to α-HNPs alone (IC\textsubscript{50} = 141.6 µg/ml). A similar effect was observed using the HepG2 cells as shown in Table 9.

To confirm the results of the MTT assay, cells were incubated with 100 µg/ml of plant extract, α-HNP, or Cfm@α-HNPs and the frequency of apoptotic cells was determined by flow cytometry. MCF7 cells treated with Cfm@α-HNPs showed the highest levels of apoptosis (20.7%) compared to cells treated with plant extract (0.67%) or α-HNP (8.39%) Fig. 14. Similar results were observed when HepG2 cells were tested. Cfm@α-HNPs and α-HNPs induced apoptosis in 17% and 7% of cells, respectively, while cells treated with the plant extract
Hydrogen peroxide scavenging (H$_2$O$_2$) assay. In the industrial process, there are some properties should be available in the catalyst as the stability of photocatalytic performance that it is very vital; enhancing catalyst life and durability simplifies operations and lowers total costs. If the catalyst has a short life cycle, it will be useless and ineffective in its photocatalytic role. One of the most effective ways that could be used to estimate the photocatalytic performance of the catalysis is the scavenging assay ability.

The human immune system has a complex composition of natural enzymatic and non-enzymatic antioxidant defenses to overcome the deleterious effects of free radicals and other oxidants. Free radicals are responsible for causing a considerable majority of diseases including ulcerative colitis, Alzheimer's disease, Parkinson's disease, atherosclerosis, alcohol-induced liver disease, cancer, cardiovascular disease, mild cognitive impairment, aging, and neural disorders. Safeguarding against free radicals may be improved by a large intake of dietary antioxidants. Substantial evidence suggests that foods containing antioxidants, and probably antioxidant nutrients, in particular, may be of significant significance in the prevention of disease. Antioxidants may benefit greatly in improving the quality of life by preventing or postponing the onset of degenerative diseases.

Human beings are indirectly exposed to H$_2$O$_2$ through the environmental surroundings at about 0.28 mg/kg/day, mainly from leaf crops. Hydrogen peroxide may reach the human body through inhalation of vapor or mist and contact with the eyes or skin. H$_2$O$_2$ is quickly decomposed into oxygen and water which may create hydroxyl radicals (•OH) that can prompt lipid peroxidation which damages the body's DNA.

Figure 15 shows the hydrogen peroxide scavenging effect of the as-biofabricated α-HNPs before and after the photocatalytic process of Cfm. It was very evident from this behavior that the scavenging rate is concentration-dependent. The IC$_{50}$ of hydrogen peroxide scavenging (%) was estimated and it was found to be 635.8 and 665.6 µg/mL of α-HNPs before and after the PCD process of Cfm respectively. As we can see the difference between α-HNPs activity before and after the photocatalytic process is less than 5%. This assures our result findings from TEM and FT-IR analysis, where the α-HNPs photocatalytic prosperity did not affect by the Cfm degradation process.

Conclusion
In the current study, green bio-fabricated α-HNPs were used in efficient degradation ability to remove the Cfm antibiotic drug substance from the contaminated industrial wastewater. The kinetic results of the photocatalytic study manifested that it was fitted to the pseudo-first-order kinetic model with R$^2$ 0.992. The optimum Cfm photocatalytic conditions were being investigated and they were found to be at a 0.4 g/L loading dose of α-HNPs over 4 h of direct sunlight irradiation at solution pH 10.5. The results revealed the possibility of using the green bio-fabricated α-HNPs as a promising and efficient photocatalytic agent in the treatment of the aquatic contaminated environment with Cfm. UV–Vis confirmed that α-HNPs had a direct transition bandgap at 3.78 eV at a maximum absorption wavelength of 362 nm. High stability of the bio-fabricated α-HNPs suspension (-68.6 mV) using zeta potential was realized. XRD, TEM, SEM, EDX, and FT-IR analyses were implemented showing synergistic results for the reusability efficiency of α-HNPs to Cfm photocatalytic achievement. Promising antibacterial activity of α-HNPs before and after the PCD process of Cfm was investigated showing highly potent ability especially against Gram-positive pathogenic issues more than Gram-negative pathogens. Interestingly, Cfm@α-HNPs showed superior anti-proliferative activity as tested by MTT assay and were able to induce apoptosis in MCF7 and HepG2 cell lines as tested by Annexin-V/PI staining using the flow cytometry technique. Hydrogen peroxide scavenging activity was conducted showing a moderated effect where the IC$_{50}$ was evaluated and it was found to be 635.8 and 665.6 µg/mL of α-HNPs before and after the PCD process of Cfm respectively.

Data availability
The data used to support the findings of this study are included in the article.
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M.F.A.: Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Validation, Writing – review & editing.G.A.G.: Conceptualization, Methodology, Software, Supervision.S.H.A.H.: Conceptualization, Methodology, Software, Supervision.M.S.S.: Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Validation, Writing – review & editing.M.A.E.: Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Validation, Writing – review & editing.M.A.I.: Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Validation, Writing – review & editing.M.M.A.M.: Conceptualization, Supervision.A.M.N.: Conceptualization, Supervision.

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