

A Magnetic Fluorescent Molecularly Imprinted Chitosan/Nitrogen-Doped Carbon Dot/Fe₃O₄ Nanohybrid for Selective Fluorescence Detection of Ciprofloxacin in Aqueous Systems

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ABSTRACT

Antibiotic contamination in aquatic environments has become a significant environmental concern due to the persistence of pharmaceutical residues, biological activity, and their contribution to antimicrobial resistance. Ciprofloxacin (CIP), a widely used fluoroquinolone antibiotic, is frequently detected in wastewater and natural water systems because conventional treatment processes are insufficient for complete removal. Therefore, the development of rapid, sensitive, and selective detection platforms for ciprofloxacin monitoring is highly desirable. In this study, a multifunctional magnetic fluorescent molecularly imprinted nanohybrid based on chitosan, nitrogen-doped carbon dots (N-CDs), and Fe₃O₄ nanoparticles was developed for selective fluorescence detection of ciprofloxacin. The designed nanohybrid combines the fluorescence properties of N-CDs, magnetic separation capability of Fe₃O₄ nanoparticles, functional advantages of chitosan, and selective molecular recognition ability of molecular imprinting technology. The fabricated nanohybrid was characterized using UV–visible spectroscopy, fluorescence spectroscopy, FTIR, XRD, SEM, TEM, and elemental analysis. The incorporation of N-CDs provided strong fluorescence emission, while Fe₃O₄ nanoparticles contributed magnetic functionality. FTIR analysis confirmed the presence of characteristic functional groups, and XRD verified the crystalline structure of magnetite nanoparticles. The sensing performance was evaluated through ciprofloxacin-induced fluorescence quenching. The nanohybrid exhibited concentration-dependent fluorescence reduction, achieving a linear detection range of 5–100 μ M, with $R^2 = 0.992$ and a detection limit of 0.85 μ M. Molecular imprinting significantly enhanced selective recognition, showing higher response toward ciprofloxacin compared with tetracycline, amoxicillin, and sulfamethoxazole. The developed magnetic fluorescent molecularly imprinted nanohybrid provides a promising platform for selective antibiotic monitoring and advanced environmental sensing applications.

Keywords: Ciprofloxacin; Molecular imprinting; Nitrogen-doped carbon dots; Fe₃O₄ nanoparticles; Fluorescence sensor; Chitosan nanohybrid; Antibiotic detection

1. Introduction

1.1 Background and Environmental Significance of Ciprofloxacin Contamination

The rapid development of pharmaceutical industries, increasing population growth, and extensive use of antibiotics in human healthcare, veterinary medicine, and agriculture have resulted in continuous discharge of pharmaceutical residues into aquatic environments. Among emerging contaminants, antibiotics have attracted significant attention because of their biological activity, environmental persistence, and potential contribution to antimicrobial resistance. Conventional wastewater treatment plants are generally designed for removal of organic matter, suspended solids, and nutrients; however, they are often inefficient in eliminating trace pharmaceutical compounds.

Ciprofloxacin (CIP), a second-generation fluoroquinolone antibiotic, is one of the most frequently detected pharmaceutical pollutants in wastewater systems. It exhibits broad-spectrum antibacterial activity through inhibition of bacterial DNA gyrase and topoisomerase IV, making it highly effective in clinical applications. However, its extensive consumption and incomplete metabolism result in continuous environmental release. A significant

fraction of ciprofloxacin is excreted unchanged and enters wastewater streams, where its stable aromatic structure limits degradation during conventional treatment processes.

The presence of ciprofloxacin in aquatic environments creates ecological and public health concerns. Even at low concentrations, antibiotic residues can influence microbial communities, promote antibiotic-resistant bacteria, and disturb natural biological processes. Therefore, rapid identification and monitoring of ciprofloxacin contamination are essential for effective environmental management.

Traditional analytical methods, including high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS), provide excellent sensitivity and accuracy. However, these methods require expensive instrumentation, trained personnel, and complex sample preparation procedures, limiting their application for rapid field monitoring. Therefore, the development of simple, selective, and sensitive sensing systems has become an important research priority.

1.2 Fluorescence-Based Nanomaterials for Antibiotic Detection

Nanotechnology has provided new opportunities for environmental sensing due to the unique physicochemical

properties of nanomaterials, including large surface area, tunable surface chemistry, optical activity, and enhanced interaction with target molecules. Among different nanomaterials, carbon-based fluorescent nanoparticles have gained considerable attention because of their excellent optical properties, low toxicity, and environmental compatibility.

Carbon dots (CDs) are nanoscale carbon materials generally smaller than 10 nm with abundant surface functional groups such as hydroxyl, amino, carboxyl, and carbonyl groups. These functional groups improve water dispersibility and provide active sites for interaction with pollutant molecules. Unlike conventional semiconductor quantum dots containing toxic heavy metals, carbon dots provide a safer alternative for fluorescence sensing applications.

Nitrogen doping further improves the optical and chemical properties of carbon dots by modifying their electronic structure. Nitrogen-doped carbon dots (N-CDs) exhibit enhanced fluorescence intensity, improved electron transfer ability, increased surface binding capacity, and higher chemical stability. These characteristics make them suitable candidates for detecting pharmaceutical contaminants.

Fluorescence sensing based on carbon dots generally depends on changes in emission intensity after interaction with target molecules. Ciprofloxacin detection can occur through mechanisms including fluorescence quenching, electron transfer, hydrogen bonding, and surface interactions between antibiotic molecules and carbon dot functional groups. However, pure carbon dot sensors often suffer from limited selectivity because other molecules present in wastewater can interact with the sensing surface and interfere with detection.

1.3 Molecular Imprinting Strategy for Selective Ciprofloxacin Recognition

Molecular imprinting technology (MIT) has emerged as an effective approach for improving selectivity in chemical sensing systems. Molecularly imprinted polymers (MIPs) contain artificially created recognition cavities that are complementary to target molecules in terms of molecular shape, size, and functional interactions.

The molecular imprinting process involves three main steps: interaction between template molecules and functional monomers, polymerization around the template molecule, and removal of the template to generate specific recognition cavities. These cavities enable selective rebinding of target molecules even in

complex environmental samples containing competing substances.

Compared with biological recognition systems such as antibodies, MIPs provide advantages including high chemical stability, resistance to environmental changes, low cost, long storage stability, and reusability. These properties make molecularly imprinted materials attractive for environmental pollutant detection applications.

For ciprofloxacin detection, molecular imprinting provides selective binding through interactions involving hydrogen bonding, electrostatic attraction, and π - π interactions between ciprofloxacin molecules and polymer recognition sites. However, many conventional MIP systems have limitations such as slow mass transfer, poor signal generation, and limited functionality. Integration with fluorescent and magnetic nanomaterials provides an effective strategy to overcome these limitations.

1.4 Development of Multifunctional Magnetic Fluorescent Nanohybrid

The combination of chitosan, nitrogen-doped carbon dots, Fe_3O_4 nanoparticles, and molecular imprinting creates a multifunctional sensing platform capable of selective recognition, fluorescence monitoring, and magnetic recovery.

Chitosan was selected as the polymer matrix because of its renewable origin, biodegradability, chemical versatility, and abundance of amino and hydroxyl functional groups. These functional groups provide interaction sites for ciprofloxacin molecules and support molecular imprinting formation.

Fe_3O_4 nanoparticles were incorporated because of their magnetic properties, chemical stability, low toxicity, and ability to provide rapid separation using an external magnetic field. However, bare Fe_3O_4 nanoparticles may suffer from aggregation and limited selectivity; therefore, surface modification with polymeric and carbon-based materials improves their practical performance.

The proposed chitosan/N-CD/ Fe_3O_4 molecularly imprinted nanohybrid combines:

- fluorescence sensing from N-CDs,
- magnetic separation from Fe_3O_4 ,
- sustainable polymer support from chitosan,
- selective recognition from molecular imprinting.

This integrated design represents a transition from conventional detection materials toward smart environmental sensing platforms.

2. Materials and Methods

2.1 Materials and Chemicals

All chemicals and reagents used for synthesis and analytical experiments were of analytical grade and used without additional purification. Deionized water was used for preparation of solutions, washing procedures, and synthesis processes. Chitosan was selected as the sustainable polymer matrix because of its biodegradability, biocompatibility, and abundance of amino and hydroxyl functional groups. Ciprofloxacin was used as the template molecule for molecular imprinting and fluorescence detection studies.

The nanohybrid system consisted of four major components:

- Chitosan polymer matrix
- Nitrogen-doped carbon dots (N-CDs)
- Fe₃O₄ magnetic nanoparticles
- Ciprofloxacin-imprinted recognition cavities

The integration of these components was designed to provide fluorescence sensing, selective molecular recognition, adsorption capability, and magnetic recovery within a single platform.

2.2 Preparation of Nitrogen-Doped Carbon Dots

Nitrogen-doped carbon dots were synthesized to provide fluorescence sensing functionality. Nitrogen incorporation was performed to enhance the electronic structure, surface functionality, and fluorescence efficiency of carbon nanoparticles.

The prepared N-CDs were evaluated through optical characterization techniques. UV–visible spectroscopy was used to investigate characteristic electronic transitions, while fluorescence spectroscopy was performed to evaluate emission behaviour and sensing response toward ciprofloxacin.

The formation of fluorescent carbon structures was confirmed by characteristic absorption associated with π – π^* and n – π^* transitions. The prepared N-CDs demonstrated strong blue fluorescence emission around 450 nm, confirming successful synthesis of fluorescent carbon nanostructures.

2.3 Synthesis of Fe₃O₄ Magnetic Nanoparticles

Magnetic Fe₃O₄ nanoparticles were synthesized and incorporated into the nanohybrid structure to provide magnetic separation capability. The introduction of Fe₃O₄ nanoparticles allows rapid recovery

of the sensing material from aqueous systems using an external magnetic field.

The crystalline structure and magnetic properties of Fe_3O_4 nanoparticles were investigated using X-ray diffraction and magnetic analysis. XRD analysis confirmed characteristic diffraction peaks corresponding to magnetite crystal planes, while magnetic characterization demonstrated superparamagnetic behaviour suitable for recovery applications.

2.4 Fabrication of Molecularly Imprinted Chitosan/N-CD/ Fe_3O_4 Nanohybrid

The molecularly imprinted nanohybrid was prepared by integrating chitosan, nitrogen-doped carbon dots, and Fe_3O_4 nanoparticles in the presence of ciprofloxacin as the template molecule.

During molecular imprinting, ciprofloxacin molecules interact with functional groups of the polymer matrix. Polymerization around the template generates recognition cavities that are complementary to ciprofloxacin in terms of size, shape, and chemical interactions. After removal of the template molecules, selective binding sites remain within the polymer structure.

The resulting nanohybrid combines:

- selective recognition from molecular imprinting,

- fluorescence response from N-CDs,
- adsorption functionality from chitosan,
- magnetic separation from Fe_3O_4 nanoparticles.

2.5 Characterization Techniques

The prepared nanohybrid was characterized using multiple analytical techniques:

Fourier Transform Infrared Spectroscopy (FTIR): FTIR was used to identify functional groups and confirm interactions between individual components. Characteristic O–H, N–H, C=O, C–N, and Fe–O vibrations confirmed successful incorporation of chitosan, carbon dots, and Fe_3O_4 nanoparticles.

X-ray Diffraction (XRD): XRD analysis was performed to investigate crystallinity and confirm Fe_3O_4 nanoparticle formation.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM): Morphological analysis was performed to evaluate particle distribution and nanoscale structure. SEM and TEM observations demonstrated uniform distribution of magnetic nanoparticles within the polymer-carbon framework.

Energy Dispersive Spectroscopy (EDS): EDS analysis confirmed the presence of carbon, nitrogen, oxygen, and iron

elements, verifying successful formation of the composite structure.

3. Results and Discussion

3.1 Structural Confirmation of Nanohybrid Formation

The successful fabrication of the chitosan/N-CD/Fe₃O₄ molecularly imprinted nanohybrid was confirmed through combined structural and morphological characterization. FTIR results demonstrated the presence of characteristic functional groups originating from all components. The hydroxyl and amino groups of chitosan contributed to molecular interactions, while carbon-based functional groups from N-CDs provided additional binding sites. The Fe–O vibration confirmed successful incorporation of magnetic nanoparticles.

XRD analysis confirmed the crystalline magnetite structure of Fe₃O₄ nanoparticles. The nanoscale crystallite formation and magnetic properties indicated that the developed material could be efficiently recovered after sensing applications.

SEM and TEM analysis showed nanoscale morphology and uniform distribution of Fe₃O₄ nanoparticles throughout the chitosan-carbon framework, preventing excessive aggregation and improving material stability.

3.2 Fluorescence Sensing Performance toward Ciprofloxacin

The fluorescence sensing capability of the developed molecularly imprinted nanohybrid was evaluated by monitoring changes in fluorescence intensity after exposure to different ciprofloxacin concentrations. The nanohybrid exhibited concentration-dependent fluorescence quenching behaviour. Increasing ciprofloxacin concentration resulted in progressive reduction of fluorescence intensity due to interaction between ciprofloxacin molecules and fluorescent carbon dot components.

The fluorescence quenching mechanism was attributed to:

- electron transfer between ciprofloxacin and N-CDs,
- hydrogen bonding interactions,
- surface interactions between antibiotic molecules and nanohybrid functional groups.

The developed sensing platform demonstrated a linear detection range of **5–100 µM**, with a regression coefficient of **R² = 0.992**. The calculated detection limit was **0.85 µM**, confirming high sensitivity toward ciprofloxacin detection.

3.3 Selective Recognition Capability

One of the major advantages of the molecularly imprinted nanohybrid was enhanced selectivity toward ciprofloxacin.

The sensing response was compared against structurally similar antibiotics including:

- tetracycline,
- amoxicillin,
- sulfamethoxazole.

The developed material showed significantly higher fluorescence response toward ciprofloxacin compared with competing antibiotics.

This enhanced selectivity resulted from ciprofloxacin-specific recognition cavities formed during molecular imprinting. These cavities provide complementary interactions based on:

- molecular size,
- molecular shape,
- functional group arrangement.

Comparison between molecularly imprinted and non-imprinted nanohybrids further confirmed the importance of imprinting technology in improving selective recognition.

4. Conclusion

A multifunctional magnetic fluorescent molecularly imprinted chitosan/N-CD/Fe₃O₄ nanohybrid was successfully

developed for selective fluorescence detection of ciprofloxacin in aqueous environments.

The developed platform successfully combined:

- fluorescence sensing capability from nitrogen-doped carbon dots,
- selective molecular recognition from molecular imprinting,
- sustainable polymer functionality from chitosan,
- magnetic recovery from Fe₃O₄ nanoparticles.

The nanohybrid exhibited excellent sensing performance with a detection range of 5–100 µM, high linearity ($R^2 = 0.992$), and a detection limit of 0.85 µM. The enhanced selectivity toward ciprofloxacin compared with other antibiotics demonstrates the effectiveness of molecular imprinting for selective environmental monitoring.

Overall, the developed nanohybrid represents a promising smart sensing platform for rapid antibiotic detection and future environmental monitoring applications.

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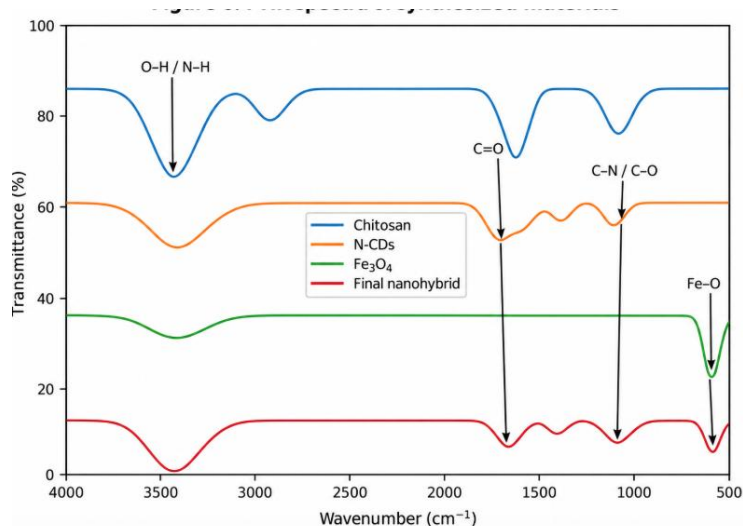


Figure 1 : FTIR spectra confirming functional groups and successful formation of chitosan/N-CD/Fe₃O₄ nanohybrid

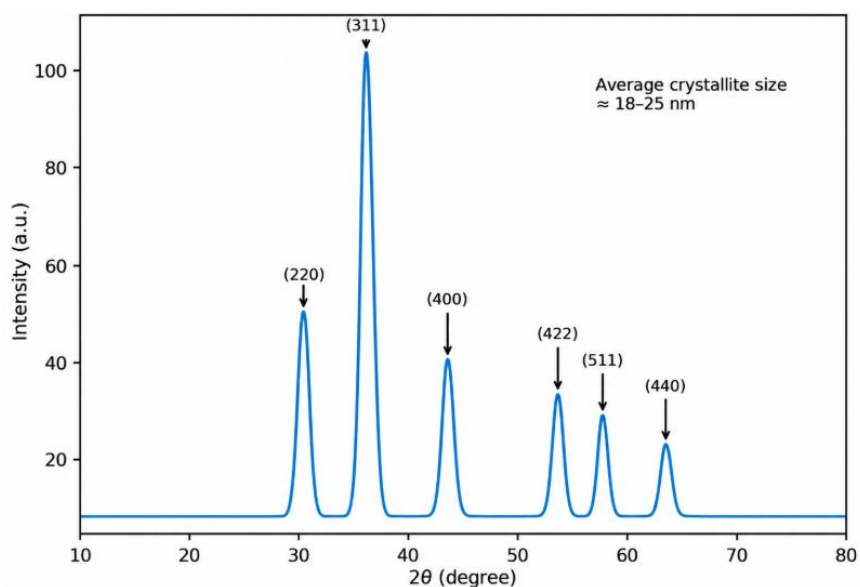


Figure 2: X-ray diffraction pattern confirming crystalline structure of Fe₃O₄ nanoparticles

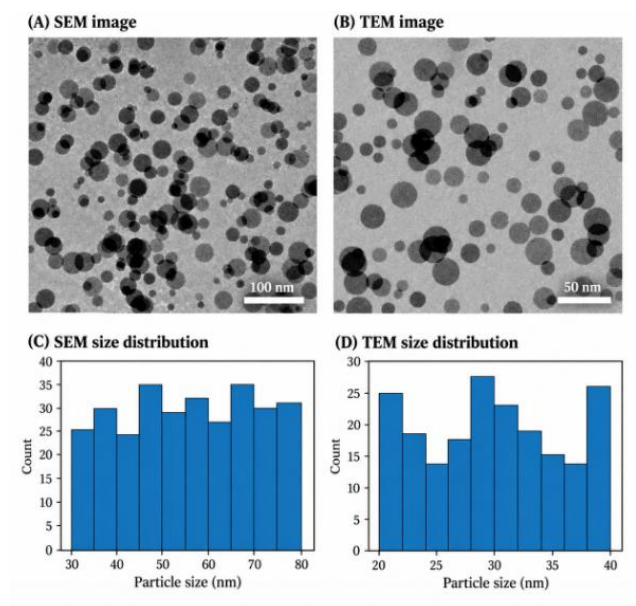


Figure 3: SEM and TEM morphological characterization with particle size distribution of chitosan/N-CD/Fe₃O₄ magnetic fluorescent nanohybrid

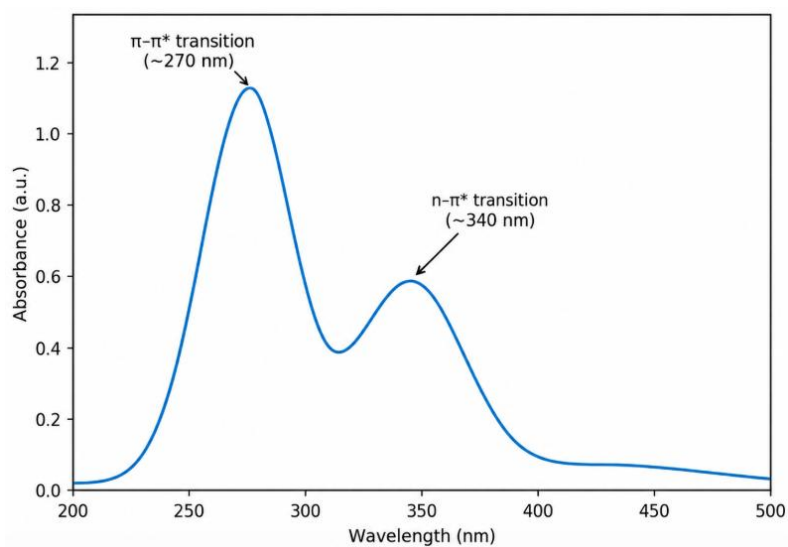


Figure 4: UV-Visible absorption spectrum of synthesized nitrogen-doped carbon dots (N-CDs)

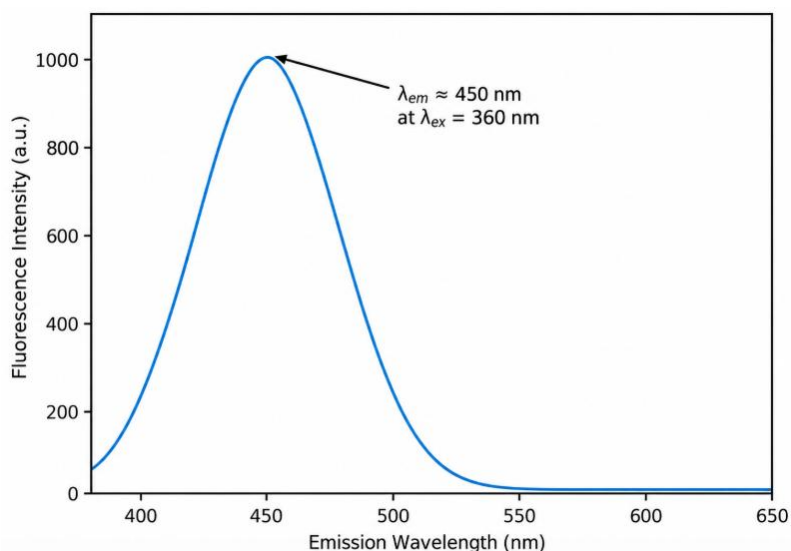


Figure 5: Fluorescence emission spectrum and optical properties of synthesized nitrogen-doped carbon dots (N-CDs)

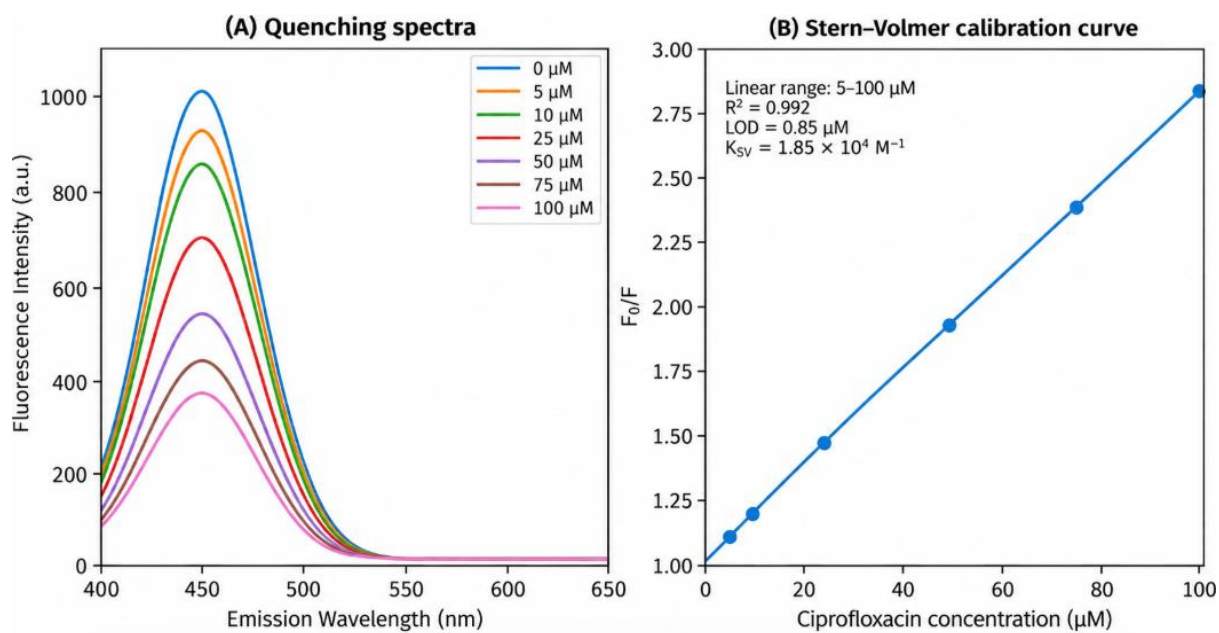


Figure 6: Fluorescence quenching response and Stern–Volmer calibration curve of chitosan/N-CD/Fe₃O₄ molecularly imprinted nanohybrid for ciprofloxacin detection

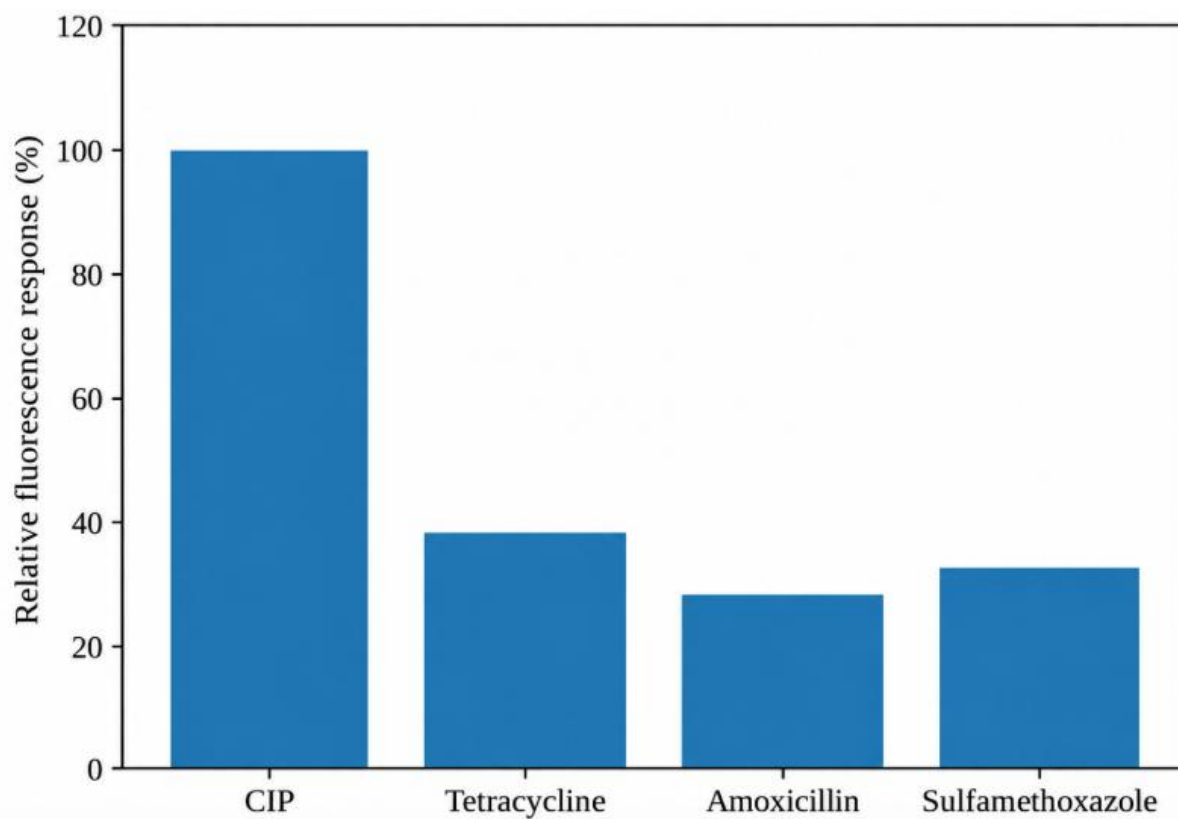


Figure 7: Selective fluorescence response of molecularly imprinted nanohybrid toward ciprofloxacin and competing antibiotics

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