

DOE-Optimized Fluorescent Sensor Based on CQDs@Ag@Cu for Sensitive Detection of Cefixime in Wastewater

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Abstract

Cefixime, a prominent member of the cephalosporin antibiotic family, is widely employed to treat various bacterial infections. Continuous monitoring of its concentration in biological fluids is essential to ensure therapeutic efficacy and minimize potential adverse effects. In this study, a novel fluorescent sensing platform was developed based on carbon quantum dots (CQDs) synthesized from *Artemisia absinthium* biomass, subsequently functionalized with copper and silver ions (CQDs@Ag@Cu) via a one-pot hydrothermal method. The synthesized nanocomposite demonstrated significant fluorescence enhancement in the presence of cefixime, which is attributed to specific interactions between the antibiotic molecules and the doped CQDs. The optimal fluorescence response was observed at pH 6.5, with minimal interference from other coexisting analytes. The sensor exhibited a linear dynamic range from 117.6 to 529.21 μM and a detection limit as low as 50.5 μM . Practical applicability was confirmed through analysis of cefixime in spiked wastewater samples, underscoring its potential utility in biomedical monitoring and clinical diagnostics. These findings endorse the eco-friendly nanoprobe as a promising tool for therapeutic drug monitoring.

Introduction

Cefixime is a widely used antibiotic essential for treating various bacterial infections ((Verma et al., 2024). However, its widespread use has raised significant concerns about environmental contamination, as residual cefixime can leach into water and soil ecosystems (Omrani et al., 2025). This accumulation may introduce antibiotics into the food chain, potentially affecting both humans and animals. The pervasive presence of antibiotics in the environment has prompted concerns over their possible health impacts, highlighting the need for continuous monitoring and precise quantification to protect public health. Nanotechnology-based methods have been successfully employed to remove several antibiotics from environmental matrices (Gorji et al., 2025; Movahedi et al., 2025; Rajabizadeh et al., 2025; Shahavi et al., 2024; Sheikh et al., 2025). Accurate measurement of antibiotic concentrations is crucial not only for ensuring proper therapeutic dosing but also for meeting regulatory requirements set by agencies such as the U.S. Food and Drug Administration (Ajmal et al., 2023). These agencies have established permissible limits for cefixime in food products and medications, which must be strictly monitored to prevent overdosing and potential toxicity. Furthermore, the excessive and improper use of cefixime has been linked to the emergence of antibiotic-resistant bacteria, a significant public health concern (Gholami et al., 2025; Nazarenko et al., 2025; Saadatmorad et al., 2025; Zandi et al., 2025). This resistance can compromise the efficacy of treatment options available for various infections, leading to increased morbidity and mortality rates (Ahmadvand et al., 2025; Saadatmorad et al., 2024; Shahavi et al., 2022). Thus, effective detection and quantification of cefixime are essential to mitigate the risks associated with its misuse and to combat the rising tide of antibiotic resistance (Ebrahimpour et al., 2009; Jahanshahi et al., 2009; Jahanshahi et al., 2015; Salehi Rad et al., 2025; Shahavi et al., 2008; Taheri et al., 2012).

Traditional detection techniques often rely on costly and complex instrumentation, along with labor-intensive sample preparation steps, which has led to growing interest in developing more accessible and user-friendly alternatives (Deymehkar et al., 2018; Karami et al., 2018; Karami and Taher, 2018; Karami et al., 2020). In this context, nanotechnology has emerged as a promising candidate. (Avvari et al., 2026; Bherade et al., 2025; Hosseini et al., 2012; Kazemeini et al., 2019; Niksefat Abatari et al., 2017; Pérez Quiñones et al., 2018; Shahavi et al., 2019; Soleymani Lashkenrai et al., 2019). Due to their exceptional optical features and nanoscale dimensions, carbon quantum dots (CQDs) offer distinct advantages in sensor fabrication and signal transduction (Karami and Taher, 2019; Kazemi et al., 2025). Among various analytical approaches, fluorescence-based methods have gained widespread use in pharmaceutical and biomedical analysis because of their high sensitivity, operational simplicity, affordability, and minimal equipment requirements. Notably, CQDs stand out among nanomaterials for their intense fluorescence emission, excellent photostability, facile synthesis, surface tunability, and low toxicity, making them highly attractive for designing efficient and biocompatible sensing systems (Pandey, 2025; Udhayakumari, 2025).

The incorporation of silver and copper into carbon quantum dots (CQDs) has recently emerged as an effective strategy for enhancing their fluorescence properties, thereby enabling the development of highly sensitive nanosensors for analyte detection, including pharmaceutical compounds like cefixime. These CQDs@Ag@Cu nanostructures possess distinctive photoluminescent features that significantly amplify the fluorescence response, making them suitable for precise and selective sensing applications (Ahmadi et al., 2019; Ahmadian et al., 2015; Salimi et al., 2018). To optimize the performance of such sensors, advanced statistical tools such as response surface methodology (RSM) and design of experiments (DOE) offer a robust framework for evaluating and fine-tuning experimental conditions (Gholami et al., 2023; Mofidian et al., 2019; Shahavi et al., 2016; Shahavi et al., 2015; Yavari et al., 2025). In particular, the use of central composite design (CCD) facilitates the assessment of both individual variables and their interactive effects, allowing for a more efficient and comprehensive optimization process compared to conventional univariate approaches. This methodology not only streamlines sensor development but also enhances its analytical reliability and applicability in real-world sample matrices. The successful integration of these strategies highlights the potential of CQDs@Ag@Cu - based fluorescent sensors in clinical diagnostics and environmental analysis.

Experimental

2.1. Reagents and materials

In this study, only analytical-grade reagents were employed, all of which were used as received. The preparation of the carbon quantum dots (CQDs) involved *Artemisia absinthium* extract as the carbon precursor, along with silver nitrate and copper nitrate, both sourced from Merck. Various salts, including calcium chloride (CaCl_2), magnesium chloride (MgCl_2), manganese(II) chloride (MnCl_2), potassium chloride (KCl), and sodium chloride (NaCl), were used to prepare supporting electrolyte solutions, while phosphate-buffered saline (PBS, pH 7.4) served as the main buffer. Bovine serum albumin (BSA) and

glucose were also obtained from Merck and utilized to examine potential interferences. A set of antibiotic standards—Cefixime, Levofloxacin, Doxycycline, Metronidazole, Cotrimoxazole, Tetracycline, Ciprofloxacin, Ceftriaxone, Azithromycin, Amoxicillin, Ofloxacin, and Clamoxin —was tested for comparative studies. The pH adjustments of all prepared solutions were carried out using 0.1 M solutions of sodium hydroxide, hydrochloric acid, sodium phosphate, and phosphoric acid. Fresh double-distilled water was used to prepare all aqueous solutions.

2.2. Instruments

Fluorescence measurements were carried out using a Perkin Elmer LS45 spectrofluorometer (USA), with the excitation wavelength set at 250 nm and emission recorded at 460 nm under ambient conditions. Structural characterization of the synthesized materials was conducted via Fourier-transform infrared (FTIR) spectroscopy (Thermo AVATAR model), scanning in the spectral range of 400–4000 cm^{-1} , with potassium bromide (KBr) employed for pellet formation. Transmission electron microscopy (TEM) was utilized to examine the internal morphology and nanostructure, using a Philips CM30 microscope operated at 300 kV. Surface morphology and particle size were further investigated by scanning electron microscopy (SEM) using a TESCAN MIRA III instrument, operated at an accelerating voltage of 15 kV.

2.3. Preparation of CQDs@Ag@Cu

Silver- and copper-doped carbon quantum dots (CQDs/Ag/Cu) were prepared using a single-step hydrothermal method. Briefly, 5 g of Artemisia absinthium extract served as the carbon source and was combined with 0.5 g of silver nitrate and an equal amount of copper nitrate. Separately, the solutions were mixed and stirred for 10 min at room temperature to form a uniform precursor solution. This mixture was then sealed in a Teflon-lined autoclave and heated at 200°C for 5 h, enabling the formation of Ag- and Cu-incorporated CQDs. After the reaction mixture cooled, it was centrifuged (10,000 rpm, 10 min) to eliminate residual solids, followed by filtration through a 0.45 μm Millipore membrane. The resulting CQDs/Ag/Cu suspension was stored in the dark at room temperature to prevent photodegradation.

2.4. Fluorescence intensity measurements

The fluorescence response of the synthesized CQDs@Ag@Cu toward different concentrations of cefixime was investigated following a defined procedure. In each experiment, 25 micrograms of the prepared nanomaterial were dispersed in 3 mL of a buffered solution adjusted to pH 6.5. Excitation was performed at 450 nm, while emission spectra were collected with the peak fluorescence monitored at 538 nm. Fluorescence intensities were recorded both prior to cefixime addition (denoted as F_0) and after its incorporation (denoted as F). The ratio F_0/F was then calculated to quantify the relationship between fluorescence variation and cefixime concentration. The limit of detection (LOD) was determined based on a signal-to-noise ratio of three, a conventional threshold used in analytical assays to identify the minimum detectable analyte concentration with confidence.

2.5. The optimization of parameters by CCD

Central Composite Design (CCD), a type of design of experiments (DOE), was applied to optimize three key factors affecting sensor performance: pH, reaction time, and temperature. The experimental plan included 20 runs combining factorial, axial, and center points with replicates to evaluate both individual and interactive effects. The parameter ranges were: pH 4.0–9.0, time 2–20 minutes, and temperature 30–60°C (Table 1). A quadratic polynomial model described the relationship between variables and sensor response:

Table 1
Experimental parameters and levels in the 20 CCD for the optimization of pH, Temp, and Time

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	pH	6.50	4.00	9.00	0.0000	Actual
B	time	11.0	2.00	20.00	0.0000	Actual
C	temperature	45.00	30.00	60.00	0.0000	Actual

$$Y = \beta_0 + (\beta_1 \times A) + (\beta_2 \times B) + (\beta_3 \times C) + (\beta_{11} \times A_2) + (\beta_{22} \times B_2) + (\beta_{33} \times C_2) + (\beta_{12} \times AB) + (\beta_{13} \times AC) + (\beta_{23} \times BC) \text{ (1)}$$

where Y is the predicted response, and A, B, and C represent pH, temperature, and time, respectively. Coefficients β denote linear, quadratic, and interaction effects. Data analysis was performed using Design-Expert software (v11.1.1.0), with ANOVA assessing model significance at $p < 0.05$. Regression and response surface analysis identified the optimal sensor conditions.

2.6. Preparation of sample

To evaluate the performance of the CQDs@Ag@Cu nanosensor in real environmental conditions, cefixime quantification was performed using spiked wastewater samples. Each 5 mL sample was first mixed with 5 mL of 10% (w/v) trichloroacetic acid to precipitate interfering components, followed by centrifugation at 4000 rpm for 30 min. The clear supernatant obtained after centrifugation was passed through a 0.45 μm Millipore membrane to eliminate remaining particulates. This filtrate was diluted 10-fold with a universal buffer adjusted to pH 6.5. For fluorescence analysis, 300 μL of the processed sample was combined with 25 μg of CQDs@Ag@Cu, and emission spectra were recorded at 538 nm under 450 nm excitation, while varying cefixime concentrations were analyzed.

Results and discussion

3.1. Characterization of CQDs@Ag@Cu

To investigate the structural and morphological characteristics of carbon quantum dots modified with silver and copper (CQDs@Ag@Cu), several advanced analytical techniques were utilized. As shown in Fig. 1, the transmission electron microscopy (TEM) image confirms that the CQDs@Ag@Cu exhibit a

nearly spherical morphology with an average diameter of roughly 5 nanometers. These small dimensions are particularly advantageous for sensing and bioimaging applications, as they benefit from quantum confinement effects that enhance their optical behavior. Figure 2 presents the FTIR spectra comparing pristine carbon quantum dots (CQDs) with their silver- and copper-doped counterparts. In panel A, the spectrum of the undoped CQDs exhibits characteristic peaks near 3150 cm^{-1} , 2200 cm^{-1} , and 1350 cm^{-1} . These absorption bands are associated with the stretching and bending modes of surface functional groups, particularly hydroxyl and carbonyl moieties. These surface functionalities are critical for improving the chemical reactivity and interaction potential of CQDs. Panel B of the same figure illustrates the FTIR spectrum after doping with Ag and Cu. While the major peaks observed in panel A remain visible, additional absorption signals appear near 900 cm^{-1} , which are indicative of newly formed Cu–C and Ag–C bonds. These spectral features provide strong evidence for the successful incorporation of silver and copper into the CQD structure.

Scanning Electron Microscopy (SEM) analysis offered valuable insights into the morphology of the synthesized nanostructures. As shown in Fig. 3a, the SEM images reveal well-dispersed, The nanoparticles exhibit an almost spherical shape with an average size ranging from 5 to 10 nm. Their consistent dimensions and low degree of aggregation indicate that the carbon quantum dots were synthesized with well-regulated morphology, a critical factor ensuring reliable and reproducible behavior in sensing-based applications. Furthermore, elemental mapping results presented in Fig. 3b demonstrate the spatial distribution of key elements on the nanoparticle surfaces. The high-intensity signals for carbon (C) and oxygen (O) confirm the organic composition of the quantum dots and further validate their surface functionalization. **Energy Dispersive X-ray Spectroscopy (EDX)** analysis, as illustrated in Fig. 3c, was employed to determine the elemental composition of the synthesized CQDs@Ag@Cu nanocomposite. The results revealed a substantial presence of carbon (39.42%) and oxygen (20.24%), affirming the organic framework of the quantum dots. Additionally, minor quantities of metallic elements—including copper (Cu), silver (Ag), and silicon (Si)—were detected, indicating successful incorporation of these ions into the nanostructure. To achieve a comprehensive insight into the spatial distribution of surface elements within the nanocomposite, line scan profiling was performed (Fig. 4). The generated elemental mapping distinctly highlights individual elements using specific color assignments: cadmium (Cd La1) is represented in light green, copper (Cu Ka1,2) in orange, sulfur (S Ka1,2) in blue, and cobalt (Co Ka1) in red. This clear separation of signals confirms the homogeneous incorporation and dispersion of the metallic elements across the CQDs@Ag@Cu surface. This detailed elemental profiling confirms the heterogeneous yet well-integrated composition of the CQDs@Ag@Cu material, reinforcing its suitability for advanced applications in biosensing and nanotechnology.

Figure 1

Figure 2

Figure 3

Figure 4

3.2 Optimization of Excitation Parameters for Enhanced Fluorescence Response

In this work, the optical behavior of the synthesized nanocomposite was systematically explored through fluorescence spectroscopy to optimize its performance in fluorescence-based sensing platforms. A series of excitation wavelengths, ranging from 250 nm to 460 nm, were evaluated to identify the condition that produces the most intense and well-defined emission spectrum. Among the tested wavelengths, excitation at 450 nm yielded the strongest and sharpest fluorescence emission, recorded at 460 nm (Fig. 5). Other excitation wavelengths failed to provide comparable spectral clarity or intensity, underscoring the critical role of precise wavelength selection in sensor performance. These findings not only contribute to the refinement of fluorescence-based detection systems but also support their broader implementation in analytical and bioanalytical technologies.

Figure 5.

3.3. Determination of the Optimal Concentration of CQDs@Ag@Cu Nanoprobe

An essential parameter influencing the performance of CQDs@Ag@Cu in fluorescence sensing is the concentration of the nanoprobe in the detection system. Excessive nanoprobe loading can lead to fluorescence quenching, whereas too low a concentration reduces the sensitivity of detection. To identify the most effective concentration, varying amounts of nanoprobe (ranging from 5 to 40 μg) were added to 3 mL of aqueous solution, and the fluorescence emission was recorded at 538 nm. The experimental results demonstrated that a concentration of 30 μg yields the highest emission intensity, suggesting this value as the optimal amount for achieving maximum sensitivity in cefixime detection (Fig. 6).

Figure 6

3.4. Statistical analysis

To systematically investigate the influence of various experimental parameters on the fluorescence response of cefixime, a Central Composite Design (CCD) approach was employed, encompassing a total of 20 experimental runs. This design enabled the efficient evaluation of both linear and nonlinear interactions while minimizing the number of required experiments. The fluorescence intensity responses, expressed as the ratio F_0/F , ranged from 1.3 to 3.1 and are detailed in Table 2. One of the significant advantages of employing CCD lies in its robustness for modeling complex response surfaces using a reduced number of experiments. Accordingly, the response data were fitted to a second-order polynomial equation, allowing the construction of a predictive model for F_0/F as a function of the input variables. Table 3 summarizes the statistical metrics of the fitted model, which exhibited a high adjusted coefficient of determination (Adj. R^2), confirming the adequacy and reliability of the model. To estimate the coefficients of the quadratic equation within the scope of Response Surface Methodology (RSM), a

multiple regression approach was utilized to analyze the experimental data. The developed predictive relationship, formulated using coded variables, can be represented by the following equation:

Table 2
Experiment runs and responses for optimizing
parameters evaluation

	Factor 1	Factor 2	Factor 3	Response 1
Run	A: pH	C: Time	B: Temp	F_0/F
1	6.5	11	45	3.01
2	9	20	30	2.99
3	6.5	26.1361	45	2.95
4	4	20	30	3.01
5	6.5	11	45	3.09
6	9	2	60	1.5
7	6.5	1	45	1.6
8	9	20	60	3.01
9	6.5	11	45	3.1
10	6.5	11	70.2269	3.1
11	6.5	11	45	3.09
12	4	2	60	1.5
13	6.5	11	45	3.09
14	4	2	30	1.3
15	10.7045	11	45	2.99
16	6.5	11	19.7731	3.05
17	6.5	11	45	3.09
18	4	20	60	2.3
19	9	2	30	1.5
20	2.29552	11	45	2.2

Table 3
Model summary statistic.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.0078	< 0.0001	0.4232	0.2222	
2FI	0.9173	< 0.0001	0.3163	-0.8829	
Quadratic	< 0.0001	0.0001	0.9130	0.5817	Suggested
Cubic	0.0001		0.9976		Aliased

$$Y = 3.9 + (0.1625 \times A) + (0.7366 \times B) - (0.0297 \times C) - (0.2233 \times A^2) - (0.5777 \times B^2) - (0.0536 \times C^2) + (0.0612 \times AB) + (0.0662 \times AC) - (0.1113 \times BC) \quad (2)$$

In this model, positive coefficients represent synergistic or enhancing effects, whereas negative coefficients reflect antagonistic or opposing influences of the variables on the response.

3.5. ANOVA

An analysis of variance (ANOVA) was conducted for the quadratic model (Eq. 2) describing the relationship between experimental variables and the fluorescence response (F_o/F) for cefixime. The results, summarized in Table 4, show that the model is statistically significant, as evidenced by a low p-value associated with the Fisher test. This indicates that the model reliably explains the observed variance in the response. The significance of individual regression coefficients was further assessed using the Student's t-test, where a high t-value coupled with a low p-value indicates a statistically significant term. The p-values also provide insight into the interaction effects among variables. According to the ANOVA results, terms A, B, A², B², and C² were found to be statistically significant ($p < 0.05$), confirming their influential role in the system. As a result, all terms retained in the model were significant, and Eq. (4) was constructed solely based on these meaningful predictors. The predicted values of F_o/F derived from this refined model are presented in Table 4. The coefficient of determination (R^2) for the model was calculated to be 0.9130, reflecting a strong correlation between the observed and predicted values. Table 5 presents the mean square values for both the regression and residual components. The high F-values and extremely low p-value ($p < 0.001$) further validate the model's overall significance at a confidence level exceeding 99% ($\alpha = 0.01$), emphasizing the model's robustness. Specifically, the linear effect of parameter B was highly significant ($p < 0.0001$), while parameter A showed moderate significance ($p = 0.0150$), and parameter C did not reach statistical significance ($p = 0.6033$). Furthermore, the squared term B² was also confirmed to be strongly significant ($p < 0.0001$), suggesting a non-linear relationship for this factor. Notably, all interaction terms included in the model were statistically significant, highlighting the complexity and interdependence among the studied variables. The final quadratic model representing the fluorescence response in terms of actual variable values is detailed in Eq. (4).

Table 4
ANOVA for response surface quadratic model for F_0/F

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8.73	9	0.9701	23.15	< 0.0001	significant
A-pH	0.3604	1	0.3604	8.60	0.0150	
B-Time	6.04	1	6.04	144.13	< 0.0001	
C-Temp	0.0121	1	0.0121	0.2880	0.6033	
AB	0.0300	1	0.0300	0.7163	0.4171	
AC	0.0351	1	0.0351	0.8381	0.3815	
BC	0.0990	1	0.0990	2.36	0.1552	
A ²	0.7251	1	0.7251	17.31	0.0019	
B ²	3.16	1	3.16	75.50	< 0.0001	
C ²	0.0418	1	0.0418	0.9981	0.3413	
Residual	0.4190	10	0.0419			
Lack of Fit	0.4133	5	0.0827	72.72	0.0001	significant
Pure Error	0.0057	5	0.0011			
Model	8.73	9	0.9701	23.15	< 0.0001	significant

Table 5
Standard deviation and R^2 of the response.

Std. Dev.	0.2047	R²	0.9542
Mean	2.57	Adjusted R²	0.9130
C.V. %	7.95	Predicted R²	0.5817
		Adeq Precision	13.0451

$$Y = 3.9 + (0.1625 \times A) + (0.7366 \times B) - (0.0297 \times C) - (0.2233 \times A^2) - (0.5777 \times B^2) - (0.0536 \times C^2) + (0.0612 \times AB) + (0.0662 \times AC) - (0.1113 \times BC) \quad (3)$$

$$Y = -0.6954 + (0.4200 \times A) + (0.2581 \times B) + (0.0170 \times C) - (0.0357 \times A^2) - (0.0071 \times B^2) - (0.00023 \times C^2) + (0.0027 \times AB) + (0.0017 \times AC) - (0.00084 \times BC) \quad (4)$$

3.6. 3D response surface plots

Three-dimensional response surface plots were generated to systematically assess the individual and combined effects of experimental factors on the fluorescence response of the CQDs@Ag@Cu sensor. These visual representations provide intuitive insights into how varying parameter combinations affect the F_0/F ratio. Notably, a plateau region was identified within the response surfaces, suggesting a stabilization of the sensor signal and indicating an optimal operational window for sensor performance. The response surface analyses, particularly those depicted in Fig. 7 and The data presented in Table 4 highlight a statistically significant interplay between pH (factor A) and temperature (factor B) influencing fluorescence intensity, despite temperature alone showing a limited direct impact. Moreover, the squared term B^2 (Time²) was identified as a critical contributor to the system's response, emphasizing the nonlinear nature of time's influence on the sensor output. Curvilinear trends observed in Fig. 7A illustrate how the response fluctuates with simultaneous changes in pH and time, confirming the significance of the AB interaction term reported in Table 4. A comparable interaction pattern was observed between temperature and time (BC interaction, Fig. 7B), although temperature alone exerted a negligible effect, as evidenced by Fig. 7C, where the F_0/F value predominantly increased with time. Optimization was systematically carried out by varying three key parameters. The investigated parameter ranges included pH (A) from 4 to 9, temperature (B) between 30 and 60°C, and reaction time (C) spanning 2.0 to 20.0 minutes. Optimal sensor performance was identified at pH 6.5, 45.0°C, and an 11.0-minute reaction duration. These conditions were derived through numerical optimization techniques aimed at maximizing the fluorescence signal within a unified experimental framework. The CQDs@Ag@Cu sensor was subsequently tested under these optimized conditions, confirming the validity and robustness of the model-derived predictions.

Figure 7

3.7. Method selectivity

The ability of the CQDs@Ag@Cu sensor to selectively detect cefixime in the presence of other substances was thoroughly examined. To evaluate this, various potentially interfering compounds—each at a concentration of 400 μ M—were tested individually under the same optimized conditions used for cefixime detection (Fig. 8). For each compound, the fluorescence response (F_0/F) was carefully recorded, allowing a direct comparison of the sensor's behavior across different analytes. The data reveal that cefixime produces a pronounced change in fluorescence intensity, distinguishing itself clearly from the other tested compounds. In contrast, the majority of interferents caused little to no alteration in the sensor signal, suggesting minimal interference. This stark contrast in fluorescence response confirms the strong selectivity of the sensor toward cefixime. As a result, the CQDs@Ag@Cu-based system proves to be a reliable and highly specific platform for the detection of cefixime, even in complex matrices containing structurally or chemically similar species.

Figure 8

3.8. Calibration

Taking advantage of the pronounced fluorescence enhancement observed at 538 nm (upon 450 nm excitation) in the presence of cefixime, the CQDs@Ag@Cu nanocomposite was developed as a highly responsive fluorescent probe for cefixime detection. To evaluate the analytical performance of this nanoprobe, different concentrations of cefixime were added to the system under optimized conditions. As depicted in Fig. 9A, a clear and proportional increase in fluorescence intensity was observed as the cefixime concentration rose, with a well-defined linear response in the range of 117.6 to 529.21 μM . This indicates the sensor's strong quantitative capability within this concentration window. For a more precise performance evaluation, a calibration plot was generated by relating the fluorescence ratio (F_0/F)—where F_0 is the intensity without cefixime and F is the intensity after its addition—to cefixime concentration. The resulting curve (shown in Fig. 9B) exhibited excellent linearity, with a correlation coefficient (R^2) of 0.9769 across the tested range. Based on a signal-to-noise ratio of 3, the limit of detection (LOD) for cefixime was calculated to be 50.5 μM . This combination of low LOD and high linearity affirms the sensitivity and precision of the CQDs@Ag@Cu -based sensor. Moreover, a comparative analysis with previously published quantum dot-based cefixime sensors demonstrates that the developed nanoprobe offers notable improvements in performance, making it a promising candidate for practical and accurate cefixime detection.

Figure 9

3.9. Interference for detection of cefixime

The selectivity of the CQDs@Ag@Cu-based probe toward cefixime was assessed by systematically investigating potential interference from various commonly occurring substances. To replicate challenging matrix conditions, a series of representative compounds, including glucose, calcium chloride (CaCl_2), manganese(II) chloride (MnCl_2), potassium chloride (KCl), sodium chloride (NaCl), phosphate-buffered saline (PBS, pH 7.4), magnesium chloride (MgCl_2), and bovine serum albumin (BSA), were examined (Fig. 9). Each of these potential interferents was evaluated at concentrations substantially exceeding that of cefixime to ensure a rigorous and reliable selectivity assessment. The results confirmed that the fluorescence response of the CQDs@Ag@Cu nanoprobe toward cefixime was minimally affected by the presence of these substances. Even at elevated levels, none of the tested interferents caused a notable deviation in signal intensity. This outstanding anti-interference capability highlights the high specificity and robustness of the developed nanoprobe. Such strong selectivity indicates that the nanoprobe can be reliably used for cefixime detection in complex biological or environmental matrices without the need for extensive sample pretreatment or purification. These findings support its practical applicability in real-world analytical scenarios (Fig. 10).

Figure 10

3.10. Application

To investigate the potential for measuring Cefixime in real samples, we selected Wastewater samples as our matrix of choice. Initially, we performed a series of preparation steps to effectively isolate the serum sample, ensuring that it was suitable for analysis. Following the isolation process, we proceeded to

dilute the serum sample by a factor of 10, utilizing a buffer with a pH of 6.5. To enhance the accuracy of our measurements, we applied the standard method of spiked addition. Specifically, we introduced a known quantity of cefixime into the serum solution under optimized experimental conditions. This spiking process was crucial, as it allowed us to assess the method's reliability and performance. After the addition, we measured the fluorescence intensity of the resulting solution, which provides valuable insights into the quantity of Cefixime present in the sample. The recorded fluorescence intensity values are summarized in Table 6. This table clearly illustrates that our method yields satisfactory results for the measurement of Cefixime, with the recovery rates falling within a range of 100.12% ,100.7 and 100.5%. Additionally, the standard deviation was found to be between 0.301, 0.625 and 0.740, indicating a high level of precision and consistency in our measurements. These findings suggest that the developed method is both effective and reliable for the quantification of Cefixime in biological samples. The superior performance of the proposed CQDs@Ag@Cu probe is highlighted by a comparison with a previously reported quantum dot-based probe (Table 7), which reveals a significantly lower limit of detection for the CQDs@Ag@Cu probe. This improved sensitivity is attributed to the enhanced sensing properties of the CQDs@Ag@Cu nanocomposite (Eskandari et al., 2017; Javaheri et al., 2025; Nakhostin Mortazavi et al., 2024; Zhang et al., 2020).

Table 6
Determination of cefixime concentration in real samples. By
fluorescence method (n = 3)

Sample	Added (μM)	found (μM)	Recovery (%)	RSD%
1	150.0	150.18	100.12	0.301
2	250.0	251.76	100.70	0.626
3	300.0	301.53	100.51	0.74

Table 7
Comparison of the performances of various sensors for detection of cefixime

Probe	Linear range	LOD	Antibiotics	[Ref]
Black Soya Bean Carbon Quantum Dots	0.1-1 μM	170 nM	cefixime	Eskandari et al., 2017
Tungsten disulfide (WS2)	00–2.500 ng/mL	45 ng/mL	cefixime	(Eskandari et al., 2017; Javaheri et al., 2025)
CdS quantum dots (QDs)	2–40 $\mu\text{g/mL}$	3.9 $\mu\text{g/mL}$	cefixime	Nakhostin Mortazavi et al., 2024
Carbon Dot	0.2×10^{-6} M to 8×10^{-6} M	0.5×10^{-7} M	cefixime	Zhang et al., 2020
CQDs/Ag/Cu	117.6 to 529.21 μM	50.5 μM	cefixime	This work

Table 6

Table 7

Conclusions

This research introduces an innovative and efficient approach for the quantitative detection of cefixime through the use of a newly developed CQDs@Ag@Cu nanocomposite. Synthesized via a hydrothermal method with zinc nitrate, silver nitrate, and *Artemisia absinthium* serving as the carbon source, this nanocomposite showcases remarkable properties upon thorough characterization using techniques such as scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). The core sensing mechanism exploits fluorescence enhancement at approximately 470 nm, which exhibits a direct correlation with cefixime concentration. Optimal results were achieved under controlled conditions of pH 6.5 and employing 25 μg of the CQDs@Ag@Cu nanoprobe at room temperature, featuring an impressive detection range of 117.6 to 529.21 μM and a limit of detection (LOD) of 50.5 μM . This method not only surpasses the sensitivity of existing cefixime detection techniques but also enhances simplicity, marking a notable advancement in the field.

Rigorous evaluation of the method's robustness against various potentially interfering substances revealed minimal interference, underscoring the exceptional selectivity of the CQDs@Ag@Cu nanoprobe for cefixime. Furthermore, successful application of this detection method on real-world samples affirms

its practical relevance. This groundbreaking technique promises significant contributions to clinical laboratories, offering both enhanced cefixime quantification and the potential for widespread implementation in diverse analytical settings.

Declarations

Ethics approval and consent to participate

The authors declare that there is no Research involving Human Participants and/or Animal

Consent for publication

All authors consent to publication of the manuscript

Competing interests

The authors declare that there is no conflict of interest.

Funding

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Author Contribution

All authors have contributed equally

Availability of data and material

The authors confirm the data of this study are available within the article.

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Figures

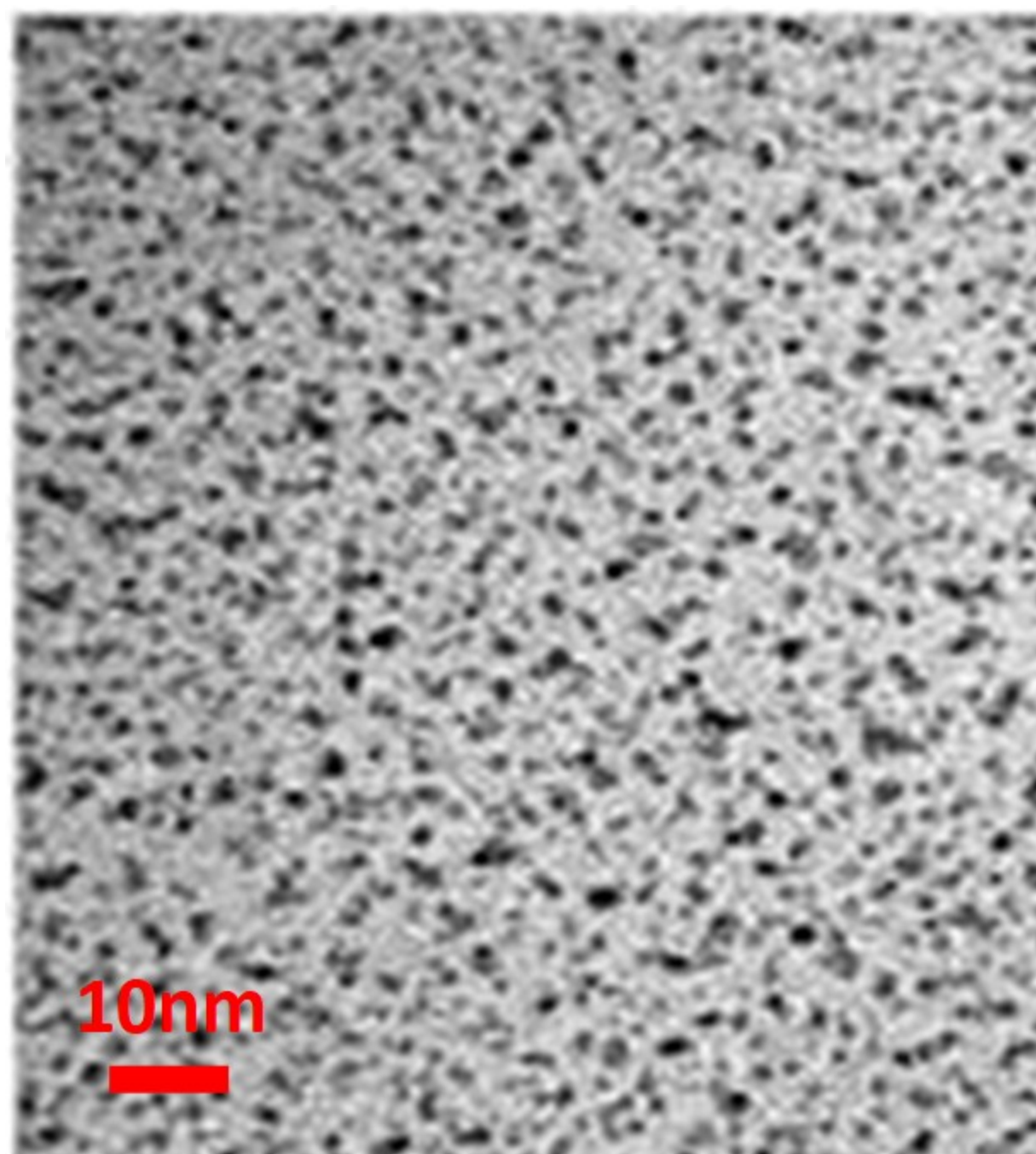


Figure 1

Electron microscope image of CQDs@Ag@Cu

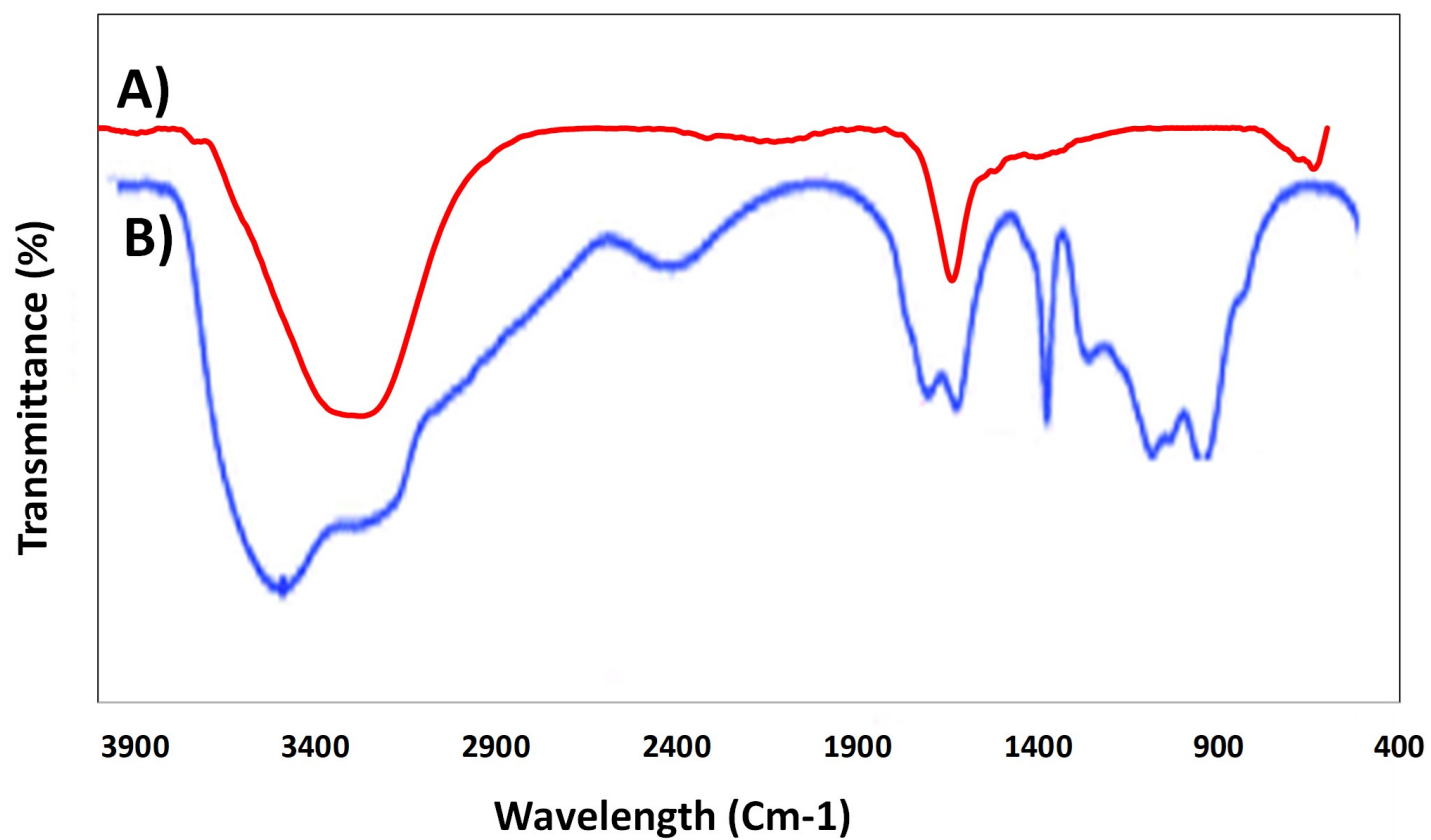


Figure 2

A) FTIR spectra of CQDs, B) FTIR spectra of CQDs@Ag@Cu

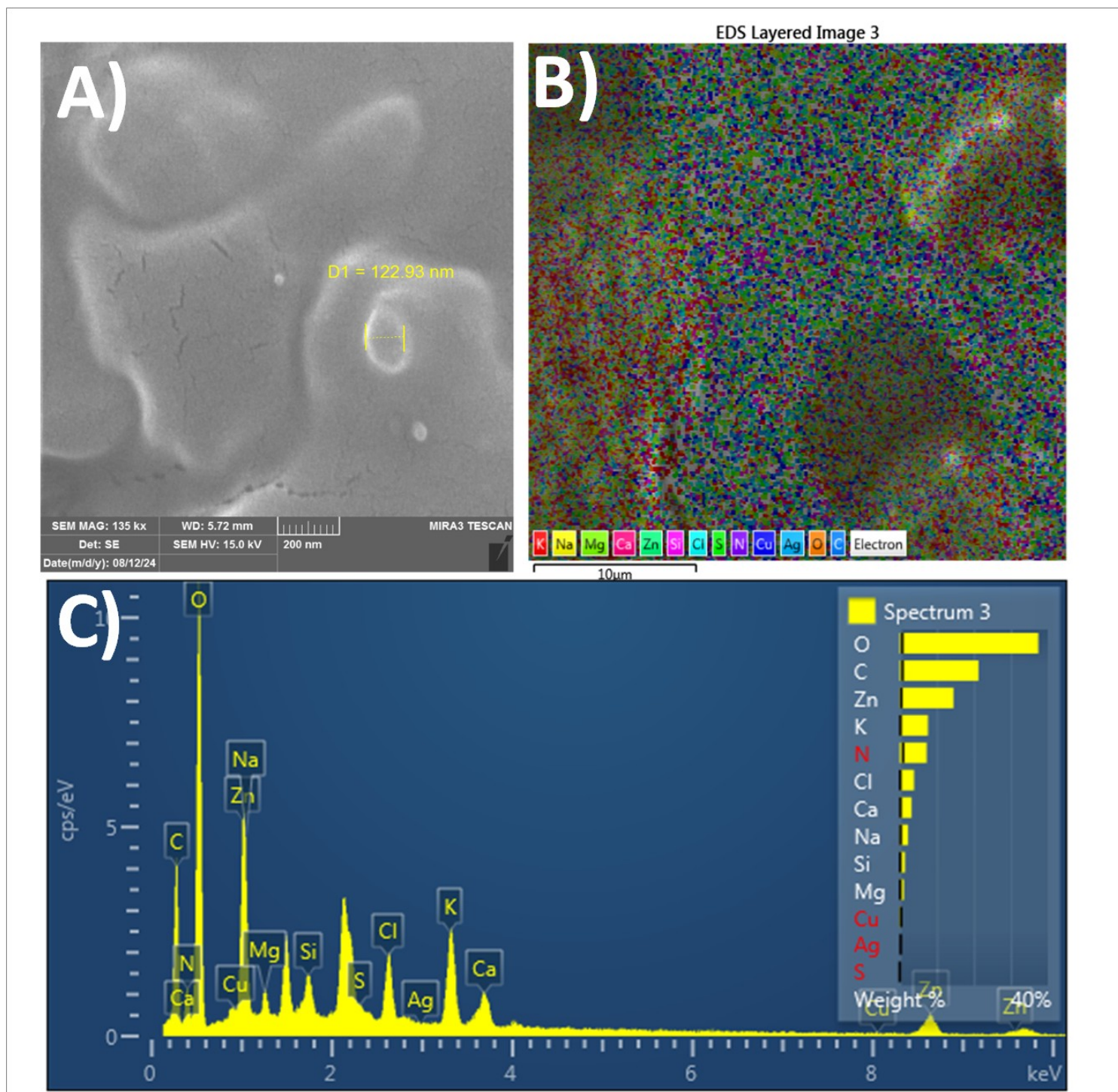


Figure 3

A) SEM images, B) mapping imaging of CQDs@Ag@Cu, C) EDS of CQDs@Ag@Cu

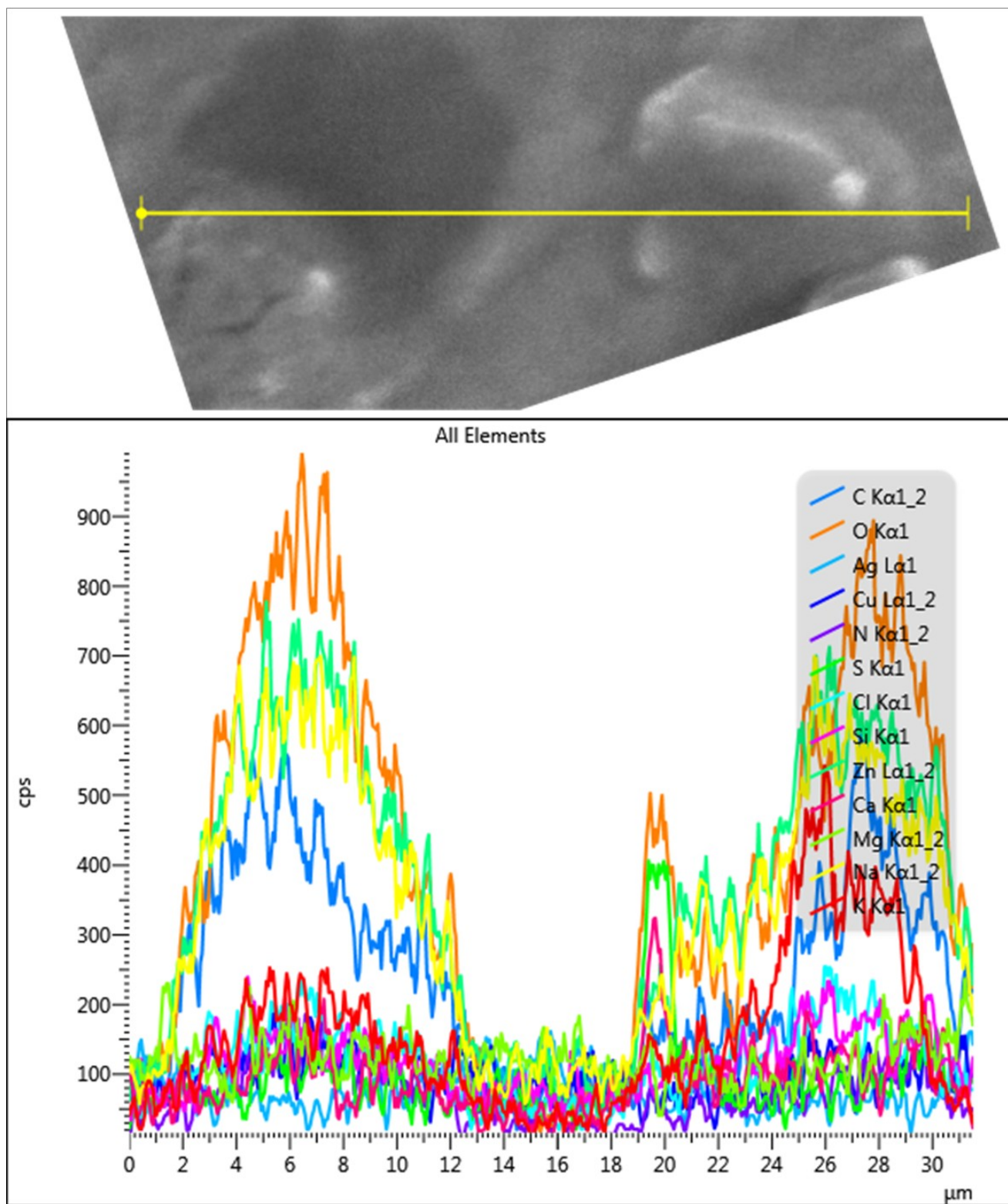


Figure 4

line scanning was each color in the resulting image corresponds to a specific element within the sample

250 nm-460 nm

440 nm is best

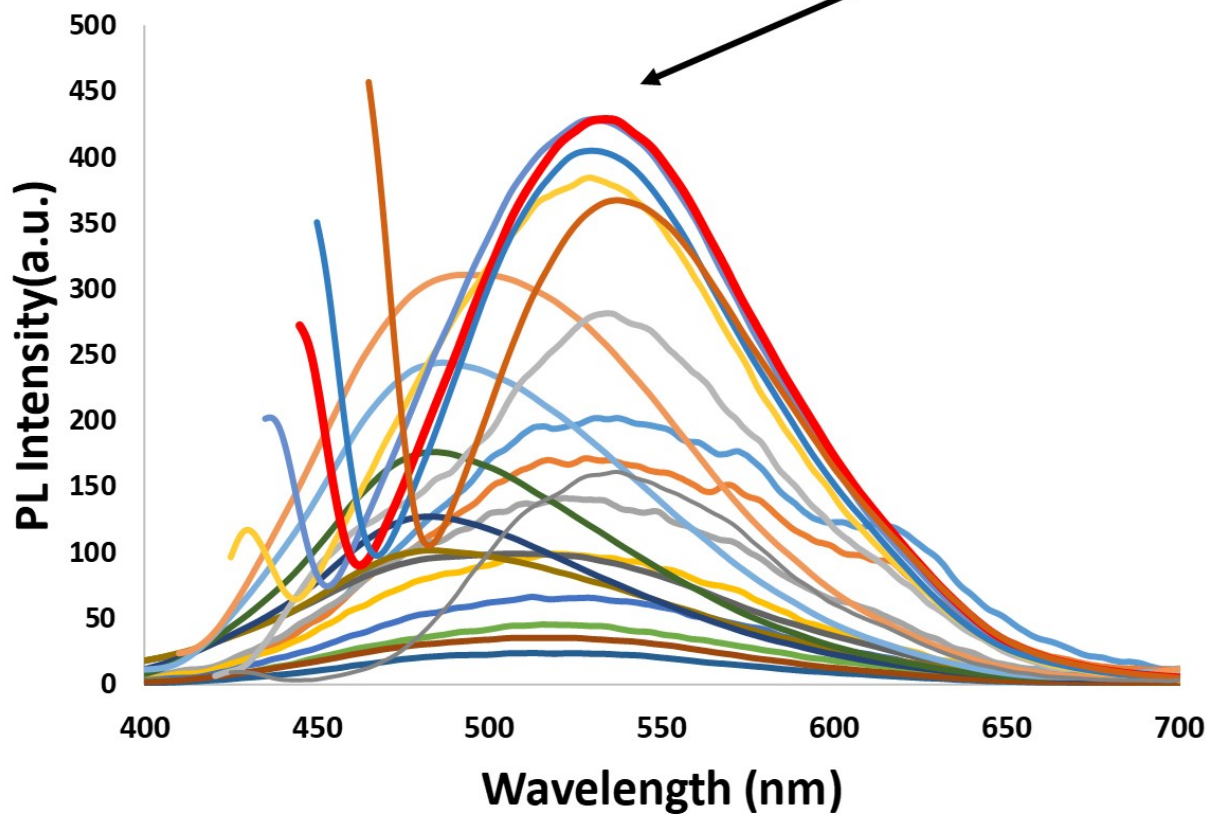


Figure 5

Fluorescence spectrum of CQDs@Ag@Cu in excitation with different wavelengths from 250 nm to 460 nm

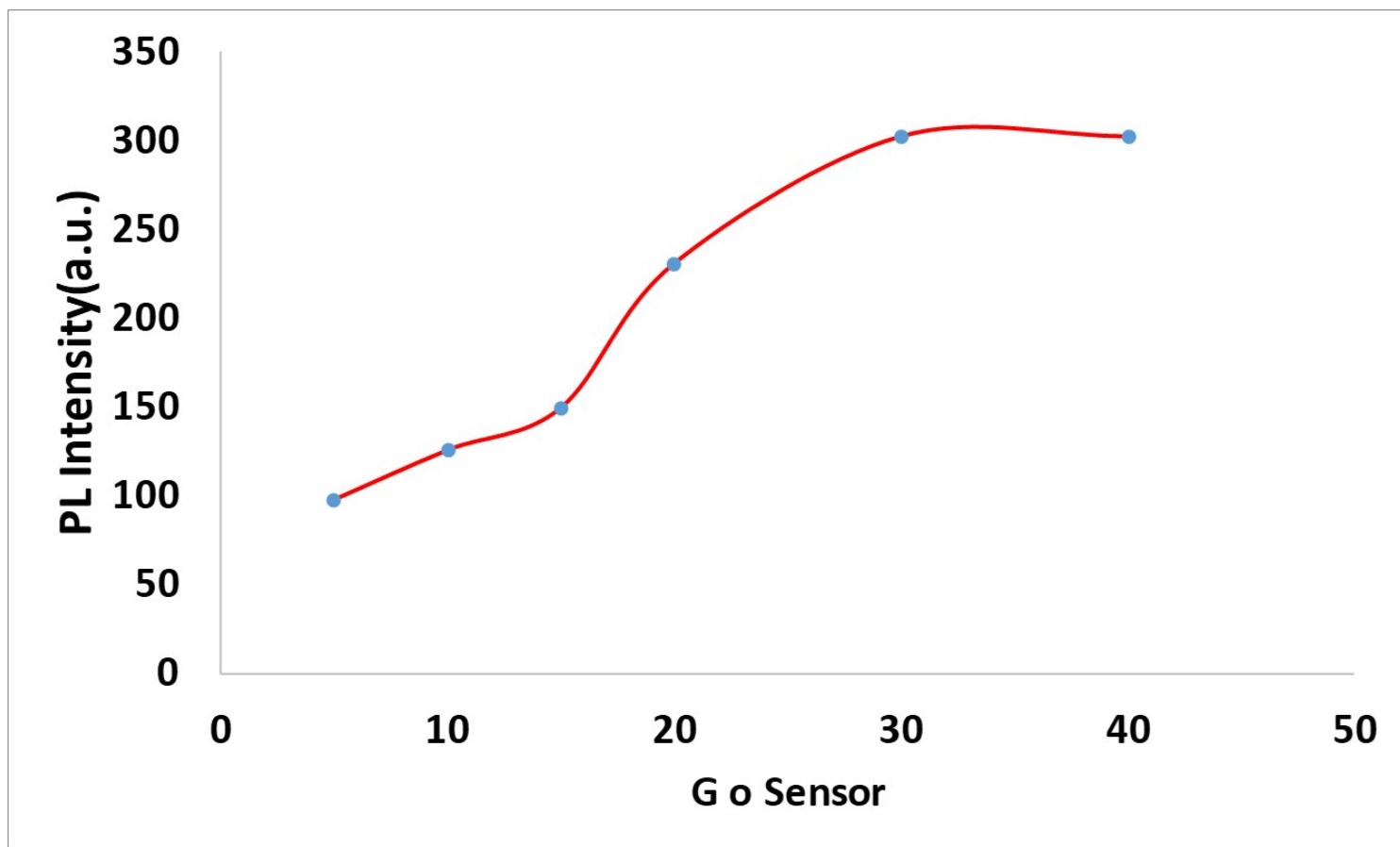


Figure 6

Effect of CQDs@Ag@Cu amount

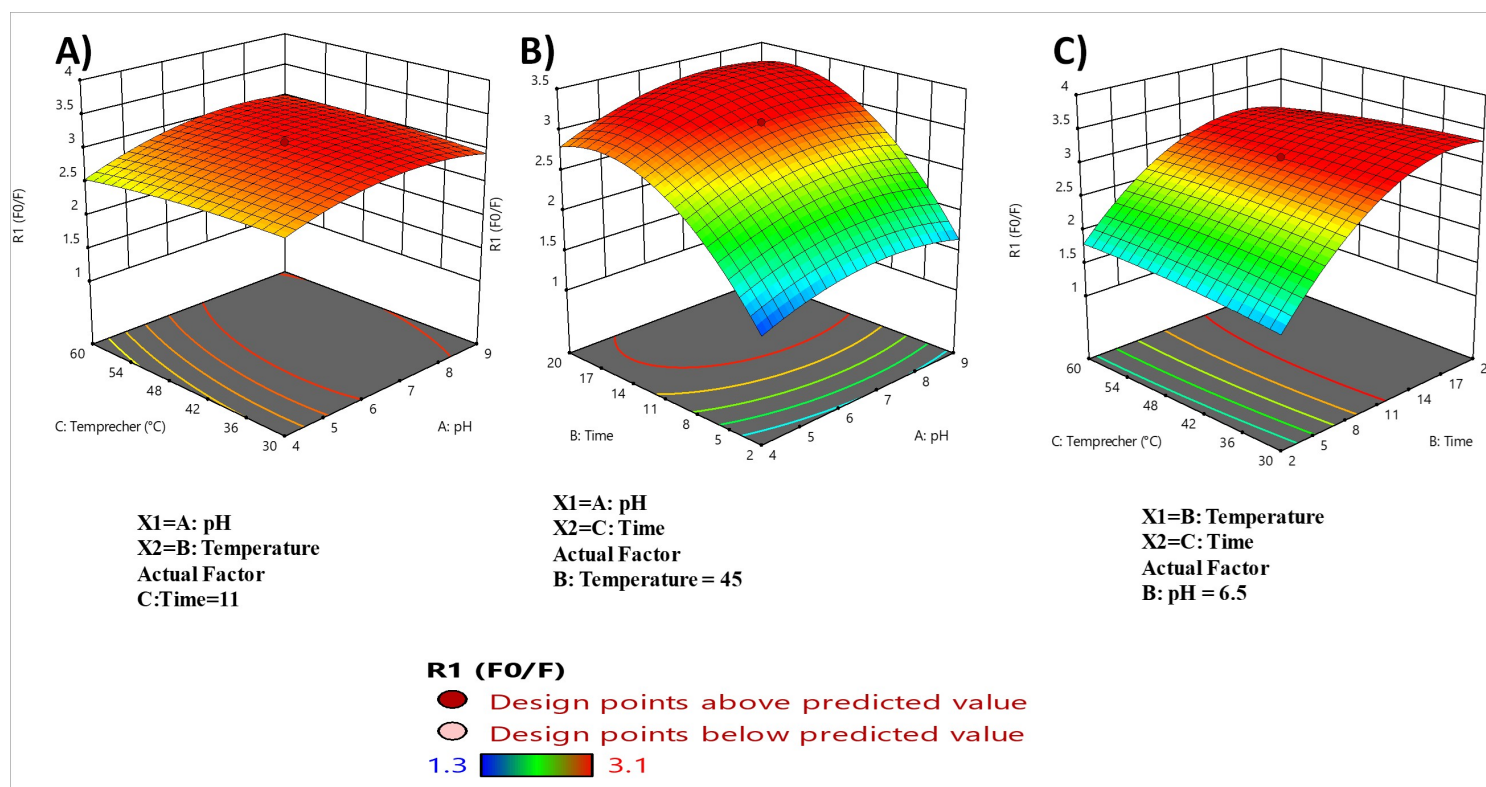


Figure 7

The 3D plot for interaction effects between pH and temperature at 11 min (A), pH and time at 45 °C (B) and temp and time at pH = 6.5 (C) for the response surface quadratic mod

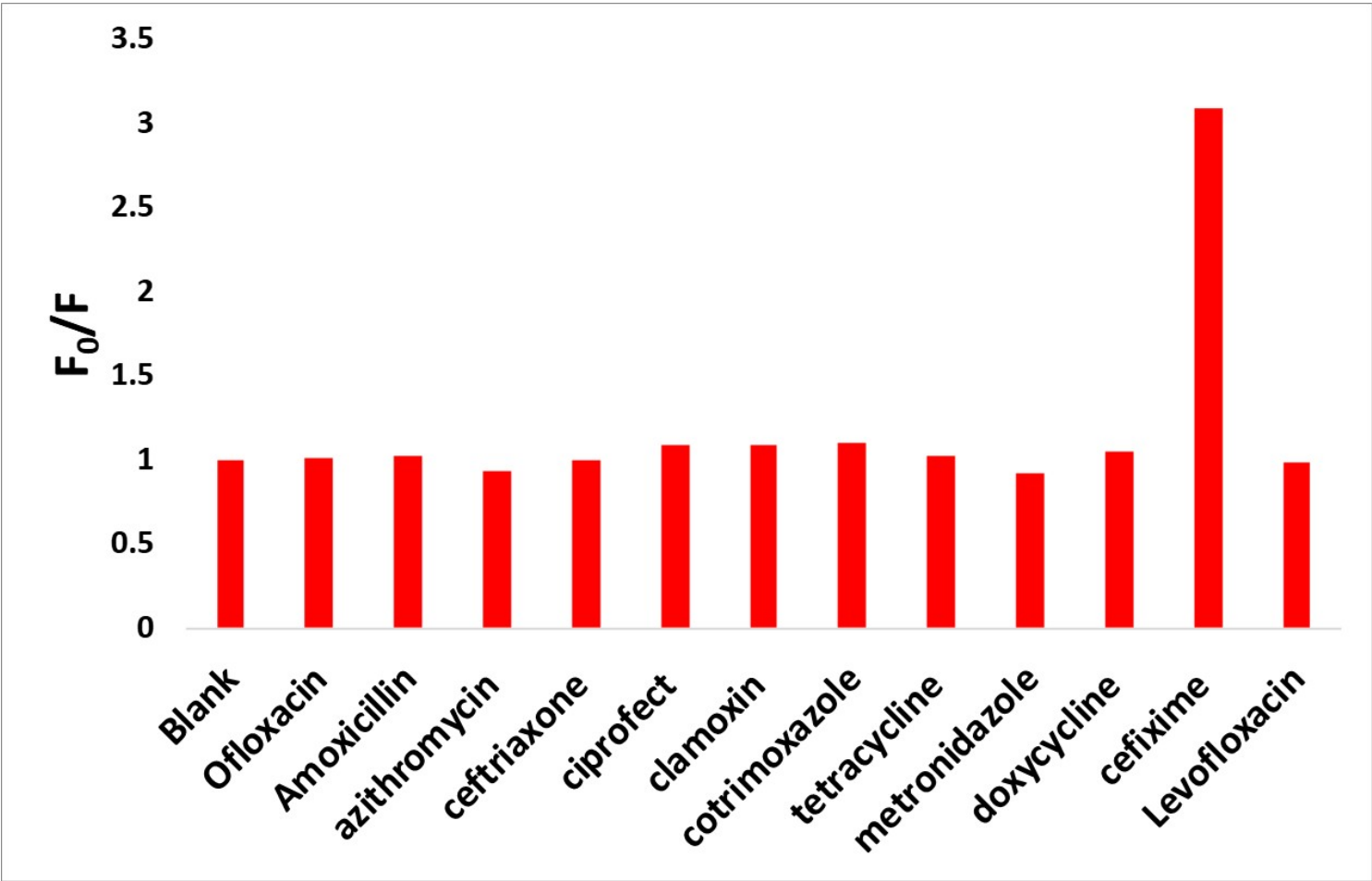


Figure 8

Fluorescence response of CQDs@Ag@Cu to various compounds

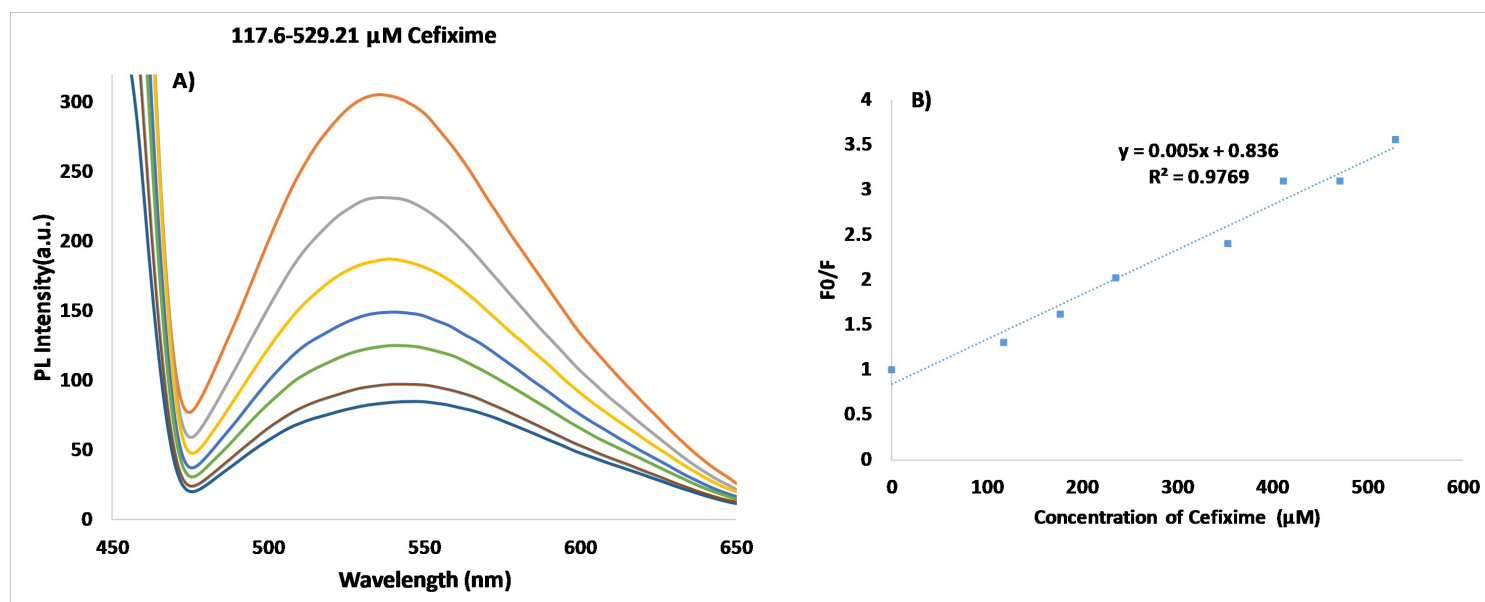


Figure 9

A) Change in the fluorescence intensity of the CQDs@Ag@Cu compound in the presence of different concentrations of cefixime from 117.6 to 529.21 μM . B) Increasing the relative sensitivity of the detection system with different concentrations of cefixime, 117.6 to 529.21 μM

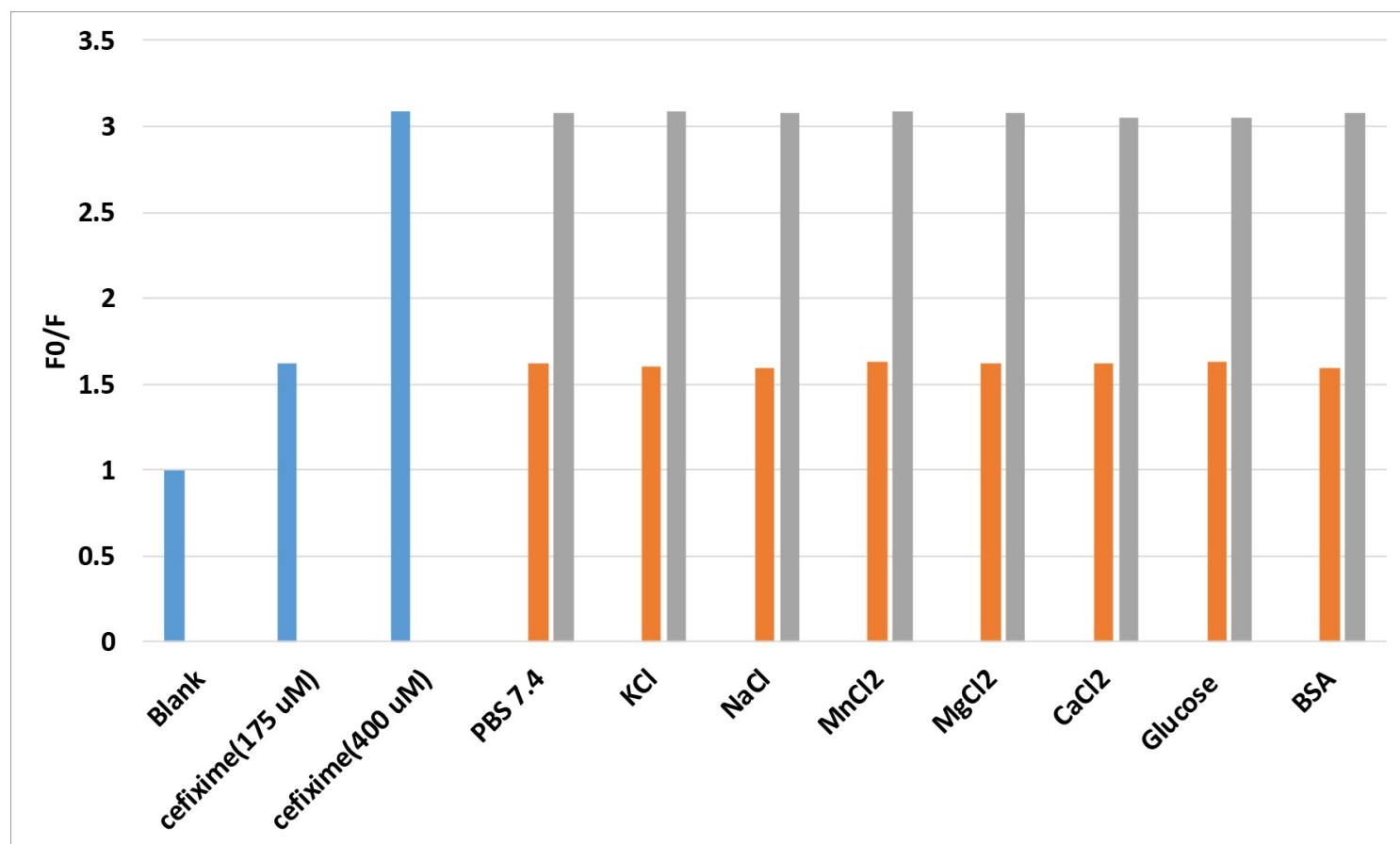


Figure 10

Fo/F ratio of CQDs@Ag@Cu in the presence of various compounds of blue rods represent cefixime alone, orange rods represent a mixture of cefixime (175 μM) with other compounds and gray rods represent a mixture of cefixime (400 μM) with other compounds.