

Eco-Friendly Carbon Quantum Dots for Fluorescent Sensing of Amoxicillin Coupled with Machine Learning and DOE

Diba Adami

Islamic Azad University

Darya Barbod

Islamic Azad University

Sara Bijari

sara.bijari75@gmail.com

Islamic Azad University

Changiz Karami

Changiz Karami, Islamic Azad University

Research Article

Keywords: Carbon Quantum Dots (CQDs), Green Synthesis, amoxicillin Detection, Machine Learning, Experimental Design, Dairy and Biological Samples

Posted Date: June 25th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8538859/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Carbon quantum dots (CQDs) synthesized via a green approach were comprehensively characterized using advanced techniques including FTIR, XRD, TEM, SEM, EDX, and fluorescence spectroscopy, confirming their nanoscale structure and functional surface groups. Experimental design was optimized using response surface methodology (RSM) and central composite design (CCD), where pH, reaction time, temperature, and sensor dosage were systematically evaluated to maximize the fluorescence quenching response (F_0/F). Statistical analysis revealed excellent model adequacy ($R^2 = 0.9834$, $\text{Adj-}R^2 = 0.9680$, $\text{Pred-}R^2 = 0.9082$), with pH, reaction time, and sensor dosage identified as the most influential parameters. Diagnostic plots verified the assumptions of linearity, normality, and homoscedasticity, ensuring predictive reliability. To further validate and enhance predictive performance, machine learning models were employed. Random Forest demonstrated superior accuracy ($R^2 \approx 0.96$) and lower error metrics compared to quadratic regression, highlighting the potential of ensemble learning in capturing nonlinear multivariate interactions. Calibration studies established two linear ranges (10–300 μM and 300–650 μM), with detection limits (LOD) of 41.47 μM and 101.78 μM and quantification limits (LOQ) of 125.66 μM and 308.43 μM , respectively. Blank precision was satisfactory ($\text{RSD} = 3.39\%$), confirming reproducibility. Interference studies showed negligible effects from common ions and biomolecules, validating the sensor's selectivity. So, the integration of structural characterization, DOE-based optimization, and machine learning evaluation underscores the robustness of the CQDs-based sensor as a sensitive, selective, and eco-friendly platform for amoxicillin detection in complex matrices.

Introduction

Amoxicillin, one of the most widely used β -lactam antibiotics, plays a crucial role in treating bacterial infections in both human medicine and veterinary practice. However, its extensive and sometimes uncontrolled use raises serious concerns for public health and the environment [1, 2]. One of the key issues associated with amoxicillin consumption is its residual presence in dairy products, particularly milk, which can enter the human food chain. These antibiotic residues may cause allergic reactions and other health complications while also contributing to the growing problem of antibiotic resistance in bacteria. Furthermore, the excessive use of amoxicillin in livestock farming leads to its release into the environment through animal waste, potentially contaminating water and soil ecosystems and affecting wildlife and human health[3, 4].

Given these concerns, it is essential to develop efficient methods for detecting and accurately measuring amoxicillin in milk and the environment. In recent years, fluorescent quantum dots (QDs) have emerged as a powerful tool for detecting pharmaceuticals and pollutants due to their exceptional optical properties, including high fluorescence intensity, photostability, and tunable emission wavelengths. In this study, quantum dots synthesized from the plant have been explored for the detection of amoxicillin in milk. Using locally sourced plants for synthesizing quantum dots is not only cost-effective and environmentally friendly but also aligns with the principles of green chemistry, promoting sustainable and eco-friendly nanotechnology approaches [5, 6].

To enhance the accuracy and sensitivity of the detection process, this study integrates two complementary scientific approaches: design of experiments (DOE) and machine learning [7]. The DOE methodology helps identify the optimal conditions for detecting amoxicillin by evaluating the effects of key factors such as pH, temperature, quantum dot concentration, and extraction parameters. Meanwhile, machine learning algorithms assist in analyzing complex data patterns, improving detection accuracy, and predicting system behavior[8].

Additionally, the structural and physicochemical properties of the synthesized quantum dots have been thoroughly analyzed using advanced characterization techniques, including Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Spectroscopy (EDX), Mapping and Line Scan imaging, Fourier Transform Infrared Spectroscopy (FTIR), and fluorescence analysis. These techniques provide valuable insights into the composition, morphology, and optical properties of the nanomaterials [9].

Overall, this study aims to develop a sensitive, selective, and sustainable method for detecting amoxicillin in milk by utilizing plant-based quantum dots, advanced analytical techniques, and machine learning models. The findings of this research can contribute to improving antibiotic monitoring in dairy products, ensuring food safety, and addressing environmental contamination caused by excessive veterinary drug use.

Experimental

2.1. Chemicals and Reagents

Amoxicillin (AMX) was obtained from a certified pharmaceutical supplier. All other chemicals, including analytical-grade solvents, acids, and bases, were purchased from Sigma-Aldrich and used without further purification. Fresh raw milk samples were collected from local dairy farms, ensuring they were free from prior antibiotic treatment. Deionized water was used for all solution preparations.

2.2. Synthesis of Plant-Based Quantum Dots (QDs)

Fluorescent quantum dots (QDs) were synthesized using an environmentally friendly approach based on *Quercus infectoria* extract. The plant extract was prepared by boiling finely ground dried leaves in deionized water, followed by filtration and centrifugation to remove impurities. The resulting extract was mixed with a carbon precursor and subjected to controlled hydrothermal treatment at 180°C for 24 hours. The obtained QDs were purified using dialysis and stored at 4°C until further use.

2.3. Characterization of Quantum Dots

The synthesized QDs were characterized using advanced analytical techniques: Transmission Electron Microscopy (TEM): Used to determine the size, morphology, and uniformity of the QDs. Energy Dispersive X-ray Spectroscopy (EDX): Used to confirm the elemental composition of the QDs. Mapping and Line Scan Imaging: Provided spatial distribution of elements within the QDs. Fourier Transform Infrared Spectroscopy (FTIR): Used to identify functional groups on the surface of the QDs. Fluorescence

Spectroscopy: Used to evaluate the optical properties and fluorescence behavior of the QDs under different excitation wavelengths.

2.4. Preparation of Standard and Sample Solutions

Stock solutions of amoxicillin (10 mg/L) were prepared in deionized water and diluted to obtain different concentrations for calibration curves. Fresh samples of raw milk were obtained from local dairies from a nearby supermarket in Kermanshah, Iran. These samples were then subjected to a series of preparatory steps following the outlined methodology. In a standard procedure, 4 mL of raw milk was combined with 10 mL of water in a 50 mL beaker, followed by the addition of 2 mL of 10% trichloroacetic acid to the diluted mixture. The mixture was then subjected to sonication at 25°C for 15 min and subsequently centrifuged at 10,000 rpm for 10 min to eliminate any sediment. The resulting supernatants were neutralized using a 30% NaOH solution and centrifuged at 20°C for 10 min to ensure the removal of any remaining debris. Furthermore, for recovery experiments, real samples were intentionally spiked with Amoxicillin, treated accordingly, and analyzed using the previously delineated procedure [10].

2.5. Fluorescence-Based Detection of Amoxicillin

For fluorescence measurements, 100 μL of the QDs solution was mixed with 300 μL of amoxicillin-containing sample and incubated for 3.5 min. Fluorescence intensity was recorded at an excitation wavelength of 320 nm, and emission spectra were analyzed to determine amoxicillin concentrations. The interaction between amoxicillin and QDs resulted in fluorescence quenching, which was correlated with the antibiotic concentration using a calibration model.

2.6. Optimization Using Design of Experiments (DOE)

To enhance detection sensitivity, a central composite design (CCD) was employed to evaluate the effects of key experimental parameters, including: pH (range: 6–8), Temperature (range: 25–45°C), QD concentration (range: 30–50 μg), Incubation time (range: 2–5 min). The response variable for optimization was based on the fluorescence intensity ratio (F_0/F), where F_0 represents the fluorescence intensity of the QDs in the absence of amoxicillin, and F represents the fluorescence intensity after interaction with amoxicillin. This ratio was used to quantify fluorescence quenching and establish a correlation with amoxicillin concentration. The DOE approach allowed for identifying the optimal conditions that maximized fluorescence quenching while ensuring reproducibility and accuracy in detection (Table 1).

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	7.00	6.00	8.00	0.0000	Actual
B	Time	3.50	2.00	5.00	0.0000	Actual
C	Temp.	35.00	25.00	45.00	0.0000	Actual
D	g of sensor	40.00	30.00	50.00	0.0000	Actual

Table 1

2.7. Machine Learning Model for Prediction

A machine learning-based predictive model was developed using experimental data to improve accuracy in amoxicillin detection. Several regression algorithms, including Support Vector Regression (SVR) and Random Forest, were trained on the dataset, with cross-validation techniques employed to evaluate model performance. The final model was used to predict amoxicillin concentrations in unknown samples, improving the robustness of the method.

2.8. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation. Data analysis was conducted using ANOVA to determine statistical significance, and correlation coefficients (R^2) were calculated to assess model accuracy.

Results and discussion

3.1. Characterization of CQDs

The morphology and size distribution of the synthesized carbon quantum dots (CQDs) were investigated using Transmission Electron Microscopy (TEM). As illustrated in Fig. 1A, the CQDs exhibit a predominantly spherical shape with uniform dispersion across the field. The particles appear well-separated, indicating minimal aggregation, which reflects the stability and quality of the synthesis process. The red scale bar in the image corresponds to 20 nm, and based on visual estimation, the average particle diameter is approximately 3 nm, consistent with the expected size range for CQDs synthesized via green methods. This nanoscale dimension confirms the successful formation of quantum-confined structures, which are essential for fluorescence behavior and sensing applications. The uniformity in particle size and shape suggests that the hydrothermal synthesis using *Quercus infectoria* extract provides a controlled environment for CQD formation. Such consistency is critical for reproducible optical properties and reliable analytical performance in subsequent detection experiments. To further investigate the surface morphology and elemental composition of the synthesized carbon

quantum dots (CQDs), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), and elemental mapping analyses were performed. Figure 1B presents the SEM micrograph of the CQDs at a magnification of 5,000 $\times$. The particles exhibit a predominantly spherical morphology with relatively smooth surfaces and uniform distribution. This morphology is indicative of successful nucleation and growth during the hydrothermal synthesis process. The absence of significant aggregation or irregular structures suggests good colloidal stability and effective surface passivation. Figure 1C shows the elemental mapping of the CQDs, revealing the spatial distribution of key elements across the sample surface. The mapping confirms the presence of carbon (C) and oxygen (O) as major constituents, along with trace amounts of potassium (K), calcium (Ca), silicon (Si), magnesium (Mg), sodium (Na), and sulfur (S). The uniform distribution of these elements supports the homogeneity of the synthesized nanomaterial and reflects the incorporation of plant-derived components during synthesis. Figure 1D displays the EDX spectrum and quantitative analysis. The dominant peaks correspond to carbon and oxygen, with weight percentages of 56.9% and 34.5%, respectively. Minor peaks for K (2.8%), Ca (2.3%), Si (1.3%), Mg (1.2%), Na (0.5%), and S (0.4%) are also observed. These trace elements likely originate from the phytochemical constituents of *Quercus infectoria* and may contribute to the unique optical and electronic properties of the CQDs. The elemental profile confirms the organic nature of the nanomaterial and supports its suitability for fluorescence-based sensing applications. To gain deeper insight into the surface chemistry and elemental distribution of the synthesized CQDs, a line scan analysis was performed using Energy Dispersive X-ray Spectroscopy (EDX). As shown in Fig. 2A, the scan was conducted along a defined horizontal path across the sample surface, capturing the spatial variation of elemental concentrations over a distance of approximately 18 μm . The resulting profile reveals the presence of carbon (C) and oxygen (O) as dominant elements, with consistently high counts per second (cps) across the scanned region. These elements form the backbone of the CQD structure and confirm the organic nature of the nanomaterial. Trace elements including sodium (Na), magnesium (Mg), silicon (Si), sulfur (S), potassium (K), and calcium (Ca) were also detected, exhibiting relatively uniform distribution along the scan line. The consistent signal intensity and smooth variation of elemental peaks suggest a homogeneous composition and well-dispersed incorporation of plant-derived constituents. This uniformity is crucial for ensuring reproducible optical and electronic behavior, particularly in fluorescence-based sensing applications. The presence of trace metals may also contribute to enhanced photoluminescence and surface reactivity, offering potential advantages for bioimaging and pharmaceutical detection. Fourier Transform Infrared Spectroscopy (FTIR) was utilized to identify surface functional groups. The spectrum (Fig. 2B) displays a broad absorption band around 3400 cm^{-1} , corresponding to O–H stretching vibrations, indicative of hydroxyl groups. A peak near 2920 cm^{-1} is attributed to C–H stretching, while a sharp band around 1700 cm^{-1} confirms the presence of carbonyl (C = O) groups. Additional bands between 1000–1300 cm^{-1} are associated with C–O stretching, suggesting alcohols, ethers, or esters on the CQD surface. Notably, no distinct peaks corresponding to metal–carbon (M–C) bonds were observed, indicating that trace metals identified in EDX are not covalently bonded but may exist as surface adsorbates. Collectively, these results confirm the successful synthesis of highly uniform, functionalized CQDs with rich oxygen-containing surface groups

and trace phytochemical elements. The structural integrity and surface chemistry of the CQDs support their application in fluorescence-based sensing of antibiotics in complex matrices.

The UV/Vis absorption spectra revealed distinct optical characteristics between CQDs synthesized from pure glucose and those derived from *Quercus infectoria* extract. As shown in Fig. 3A, the glucose-based CQDs (curve A) exhibited a sharp absorption peak at lower wavelengths, typical of π - π^* transitions in sp^2 -hybridized carbon domains. In contrast, the Quercus-derived CQDs (curve B) displayed a broader peak shifted toward higher wavelengths, indicating a red-shift associated with enhanced conjugation and the presence of diverse oxygen-containing functional groups such as hydroxyl and carbonyl moieties. This spectral broadening and shift suggest a richer distribution of surface states and defect-related transitions, which are consistent with the FTIR-confirmed surface chemistry and the phytochemical complexity of the plant extract. The broader absorption profile of Quercus-derived CQDs implies improved light-harvesting capabilities and stronger excitation–emission coupling, which are advantageous for fluorescence-based sensing. The absence of sharp metal–carbon absorption features confirms that trace metals identified via EDX are not covalently integrated into the carbon lattice but may exist as surface-bound species. Overall, the UV/Vis analysis supports the superior optical tunability and functional diversity of the green-synthesized CQDs, reinforcing their suitability for sensitive antibiotic detection in complex matrices. The fluorescence emission behavior of the synthesized CQDs was evaluated under various excitation wavelengths to determine their optimal photoluminescent response. As illustrated in Fig. 3B, the CQDs exhibited weak and poorly defined emission spectra at lower excitation wavelengths (below 300 nm), indicating suboptimal excitation efficiency in that range. However, upon excitation at 320 nm, the CQDs displayed a pronounced and well-resolved emission peak centered around 425 nm, representing the highest fluorescence intensity observed across the tested range. This excitation–emission pairing suggests that the CQDs possess a specific excitation threshold for maximum quantum yield, likely governed by their surface functional groups and core electronic structure. The sharp emission at 425 nm confirms the presence of stable radiative recombination pathways, while the diminished intensity at higher excitation wavelengths reflects reduced overlap with the absorption band and possible non-radiative losses. The strong fluorescence response at 320/425 nm supports the suitability of these CQDs for sensing applications, particularly in environments requiring high signal-to-noise ratios. The defined spectral features and excitation-dependent behavior further validate the structural uniformity and surface passivation achieved through green synthesis using *Quercus infectoria* extract.

Figure 1

Figure 2

Figure 3

3.2 Selectivity Evaluation

To assess the selectivity of the synthesized CQDs toward amoxicillin detection, fluorescence quenching experiments were conducted in the presence of various structurally related antibiotics at a fixed concentration of 300 μM . As shown in Fig. 4A, the CQDs exhibited a significantly higher fluorescence quenching response upon interaction with amoxicillin compared to other tested analytes, including amoxicillin, cefixime, ciprofloxacin, and chloramphenicol. This pronounced quenching effect suggests a preferential interaction between amoxicillin molecules and the surface functional groups of the CQDs, likely facilitated by π - π stacking, hydrogen bonding, and charge-transfer mechanisms. The minimal response observed for other antibiotics confirms the sensor's high selectivity, which is critical for reliable detection in complex biological and food matrices. The selectivity profile underscores the potential of Quercus-derived CQDs as targeted fluorescent probes for amoxicillin monitoring, minimizing false positives and enhancing analytical accuracy in real-world applications.

Figure 4

3.3. Experimental Design and Model Evaluation

To optimize the fluorescence quenching response (F_0/F) for amoxicillin detection using CQDs, a central composite design (CCD) was employed, evaluating the effects of four key factors: pH (A), temperature (B), incubation time (C), and sensor dosage (D). As shown in Table 2, 30 experimental runs were conducted, systematically varying these parameters to explore their individual and interactive effects on the sensor's performance. The F_0/F values ranged from 1.10 to 1.65, indicating significant variation in fluorescence quenching under different conditions. Maximum responses ($F_0/F = 1.65$) were observed in runs with higher temperature (45°C), longer incubation times (5–6.5 min), and elevated sensor dosages (50–60 μg), particularly at neutral to slightly alkaline pH (6–8). In contrast, lower responses were associated with acidic pH (5), short incubation (0.5–2 min), and minimal sensor dosage (20–30 μg), confirming the importance of these factors in enhancing analyte–sensor interaction. To statistically evaluate the model fit and predictive power, regression models of increasing complexity were compared, as summarized in Table 3. The quadratic model demonstrated superior performance, with an adjusted R^2 of 0.9680 and predicted R^2 of 0.9082, indicating excellent agreement between experimental and predicted values. The lack-of-fit p-value of 0.1032 suggests that the model adequately captures the data variability without significant unexplained error. In contrast, the linear and 2FI models showed lower R^2 values and significant lack-of-fit, while the cubic model, despite a high adjusted R^2 (0.9861), was aliased due to overfitting and limited degrees of freedom. Based on these results, the quadratic model was selected as the most appropriate for response surface analysis and optimization. This model enables accurate prediction of fluorescence quenching behavior across the experimental domain and supports the identification of optimal sensing conditions for amoxicillin detection.

Table 2
Experiment runs and responses for optimizing parameters evaluation

Run	Factor 1	Factor 2	Factor 3	Factor 4	Response 1
	A: pH	C: Time	B: Temp	D: g of sensor	F ₀ /F
1	7	3.5	35	40	1.45
2	5	3.5	35	40	1.24
3	6	5	45	50	1.65
4	6	2	25	30	1.22
5	7	3.5	35	20	1.4
6	6	2	25	50	1.22
7	8	5	45	50	1.65
8	6	2	45	50	1.25
9	7	3.5	35	40	1.45
10	8	5	25	30	1.43
11	7	3.5	35	40	1.45
12	8	2	25	50	1.45
13	6	2	45	30	1.25
14	7	3.5	35	60	1.65
15	6	5	25	30	1.45
16	8	5	45	30	1.45
17	8	5	25	50	1.65
18	7	3.5	35	40	1.48
19	8	2	45	50	1.4
20	7	3.5	35	40	1.48
21	6	5	45	30	1.44
22	6	5	25	50	1.65
23	7	3.5	25	40	1.48
24	7	3.5	35	40	1.48
25	7	3.5	55	40	1.48

Run	Factor 1	Factor 2	Factor 3	Factor 4	Response 1
	A: pH	C: Time	B: Temp	D: g of sensor	F ₀ /F
26	7	0.5	35	40	1.1
27	9	3.5	35	40	1.45
28	7	6.5	35	40	1.65
29	8	2	45	30	1.35
30	8	2	25	30	1.45

Table 3
Model summary statistic.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.0011	0.7485	0.6623	
2FI	0.0098	0.0036	0.8530	0.8111	
Quadratic	< 0.0001	0.1032	0.9680	0.9082	Suggested
Cubic	0.0863	0.2779	0.9861		Aliased

Table 2

Table 3

3.4. ANOVA

To evaluate the statistical robustness of the quadratic model constructed for predicting the fluorescence quenching response (F_0/F), an Analysis of Variance (ANOVA) was performed, and the results are summarized in Table 4. The overall model was highly significant, with an **F-value** of 63.59 and a corresponding p-value < 0.0001, confirming that the probability of this model arising due to random noise is less than 0.01%. This demonstrates that the quadratic model provides a reliable fit to the experimental data. Among the independent variables, reaction time (B) was identified as the most influential factor, with an exceptionally high F-value of 513.37 and $p < 0.0001$, highlighting its critical role in determining sensor response. pH (A) and sensor dosage (D) also showed strong statistical significance ($F = 77.64$ and 117.87 , respectively; $p < 0.0001$), underscoring the importance of solution chemistry and material concentration in optimizing fluorescence quenching. In addition, interaction terms such as AB (pH $\times$ time) and BD (time $\times$ sensor dosage) were highly significant ($p < 0.0001$), reflecting synergistic effects between these parameters. Quadratic terms including A^2 (pH), B^2 (time), and D^2 (sensor dosage) were statistically significant as well, confirming the presence of non-linear relationships that must be considered in the optimization process. In contrast, temperature (C) and its associated interaction and quadratic terms (AC, AD, BC, CD, C^2) exhibited p-values much greater than 0.05, indicating negligible

influence within the studied range. While these terms were retained in the model for completeness and hierarchical consistency, their lack of statistical significance suggests that temperature does not substantially affect the fluorescence response under the tested conditions. The lack-of-fit test yielded a p-value of 0.1032, which is not significant relative to pure error. This result is desirable, as it indicates that the model adequately describes the experimental data without systematic deviation. Taken together, the ANOVA results confirm that the quadratic model is statistically robust, with critical contributions from pH, reaction time, sensor dosage, and their interactive and squared effects. This reinforces the utility of CCD-RSM in accurately modeling and optimizing the sensing performance of CQDs for amoxicillin detection.

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

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.5993	14	0.0428	63.59	< 0.0001	significant
A-pH	0.0523	1	0.0523	77.64	< 0.0001	
B-Time	0.3456	1	0.3456	513.37	< 0.0001	
C-Temp.	0.0005	1	0.0005	0.8030	0.3844	
D-g of sensore	0.0794	1	0.0794	117.87	< 0.0001	
AB	0.0324	1	0.0324	48.13	< 0.0001	
AC	0.0020	1	0.0020	3.01	0.1034	
AD	0.0002	1	0.0002	0.3342	0.5718	
BC	0.0006	1	0.0006	0.9284	0.3506	
BD	0.0380	1	0.0380	56.48	< 0.0001	
CD	0.0001	1	0.0001	0.1485	0.7053	
A ²	0.0250	1	0.0250	37.20	< 0.0001	
B ²	0.0141	1	0.0141	20.90	0.0004	
C ²	0.0008	1	0.0008	1.11	0.3077	
D ²	0.0063	1	0.0063	9.39	0.0079	
Residual	0.0101	15	0.0007			
Lack of Fit	0.0087	10	0.0009	3.24	0.1032	not significant
Pure Error	0.0013	5	0.0003			
Cor Total	0.6094	29				

Table 4

3.5. 3D response surface plots

To visualize the interactive effects of experimental parameters on the fluorescence quenching efficiency (F_0/F), three-dimensional response surface plots were constructed based on the fitted quadratic model. These plots provide a comprehensive understanding of how pairs of variables influence the sensor's performance, while other factors are held constant at their central levels. The response surfaces reveal both linear and nonlinear interactions, supporting the statistical findings from ANOVA and guiding the identification of optimal sensing conditions. Figure 5A illustrates the interaction between pH (A) and reaction time (B). The surface exhibits a pronounced curvature, with maximum F_0/F values observed at neutral to slightly alkaline pH (6–8) and extended reaction times (4–6 min). This behavior reflects the enhanced electrostatic and hydrogen bonding interactions under favorable protonation conditions and sufficient analyte–sensor contact time. Figure 5B presents the combined effect of pH (A) and sensor dosage (D). The response increases with both parameters up to a saturation point, beyond which further increases yield marginal improvements. This suggests that optimal surface coverage and pH-dependent charge distribution are critical for maximizing fluorescence quenching. Figure 5C shows the interaction between reaction time (B) and sensor dosage (D). A steep gradient is observed, indicating that longer incubation and higher CQD concentrations significantly enhance analyte accessibility and signal suppression. The synergistic effect between these two factors confirms their dominant role in controlling sensor sensitivity. Figure 5D explores the relationship between pH (A) and temperature (C). The surface remains relatively flat along the temperature axis, consistent with the ANOVA results indicating the negligible influence of temperature within the tested range. However, pH continues to exert a strong effect, reinforcing its importance in modulating surface charge and binding affinity. Figure 5E depicts the interaction between reaction time (B) and temperature (C). Similar to the previous plot, temperature variation does not significantly alter the response, while time remains a key determinant of quenching efficiency. Figure 5F illustrates the combined effect of sensor dosage (D) and temperature (C). The response surface is nearly planar along the temperature axis, confirming minimal thermal sensitivity. In contrast, increasing sensor dosage leads to a marked improvement in F_0/F , validating its direct contribution to analyte capture and fluorescence modulation. Collectively, these response surface plots confirm that pH, reaction time, and sensor dosage are the most influential parameters in the sensing system, while temperature plays a negligible role under the studied conditions. The visualizations support the predictive capability of the quadratic model and demonstrate the effectiveness of CCD-RSM in optimizing fluorescence-based detection of amoxicillin using green-synthesized CQDs.

Figure 5

3.6. Model Adequacy and Validation

The statistical parameters presented in Table 5 confirm the robustness and predictive capability of the developed quadratic model. The standard deviation (0.0259) and coefficient of variation (C.V. = 1.80%)

are both very low, indicating minimal dispersion of residuals and high reproducibility of the experimental data. The mean response value of 1.44 further supports the consistency of the dataset. The model determination coefficient ($R^2 = 0.9834$) demonstrates that more than 98% of the variability in the fluorescence quenching response is explained by the fitted model. The close agreement between the adjusted R^2 (0.9680) and the predicted R^2 (0.9082), with a difference less than 0.2, confirms that the model is neither overfitted nor underfitted and possesses strong predictive reliability across the design space. This alignment validates the suitability of the quadratic model for response surface optimization. Furthermore, the adequate precision value of 29.3653 is far greater than the threshold of 4, signifying an excellent signal-to-noise ratio. This high value indicates that the model can effectively discriminate between significant and insignificant effects, ensuring reliable navigation of the experimental domain. Collectively, these statistical indices confirm that the quadratic model is statistically sound, highly predictive, and suitable for guiding the optimization of sensing parameters in amoxicillin detection.

Table 5			
Standard deviation and R^2 of the response.			
Std. Dev.	0.0259	R^2	0.9834
Mean	1.44	Adjusted R^2	0.9680
C.V. %	1.80	Predicted R^2	0.9082
		Adeq Precision	29.3653

Table 5

3.7 Model Diagnostics and Assumptions Verification

To ensure the validity and reliability of the fitted quadratic regression model, a series of diagnostic plots were examined, as shown in Fig. 6. These plots assess key statistical assumptions including linearity, normality, homoscedasticity, and the absence of influential outliers. The Residuals **vs** Fitted plot (Fig. 6A) displays a random scatter of residuals around the horizontal axis, with no discernible pattern or curvature. This indicates that the model captures the underlying relationship between predictors and response adequately, and the assumption of linearity is satisfied. The Normal Q-Q plot (Fig. 6B) shows that the residuals closely follow the reference line, confirming that the error terms are approximately normally distributed. This supports the validity of parametric inference and the use of ANOVA for model evaluation. The Scale-Location plot (Fig. 6C), which presents the square root of standardized residuals against fitted values, reveals a relatively uniform spread across the range. This suggests that the variance of residuals remains constant (homoscedasticity), and no transformation of the response variable is required. Finally, the Residuals vs Leverage plot (Fig. 6D) was used to identify influential data points. All observations fall within the Cook's distance boundaries, indicating that no single data point exerts undue influence on the model's predictions. This confirms the stability and robustness of the fitted model across the experimental domain. Collectively, these diagnostic evaluations affirm that the regression model meets the core statistical assumptions required for reliable prediction and

optimization. The absence of systematic bias, non-normality, or influential outliers reinforces the credibility of the CCD-RSM approach in modeling the fluorescence quenching behavior of CQDs.

Figure 6

3.8. Machine Learning Model Evaluation for Amoxicillin Prediction

To assess the predictive performance of different regression models for amoxicillin detection, two approaches—Random Forest and Quadratic Polynomial Regression—were evaluated using key statistical metrics: coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE). Figure 7A presents a three-dimensional performance comparison of the models in R^2 –RMSE–MAE space. The Random Forest model (green point) is positioned in the optimal region, characterized by a high R^2 and low error metrics, indicating strong predictive accuracy and robustness. In contrast, the Quadratic Regression model (blue point) shows slightly lower performance, with reduced R^2 and elevated RMSE and MAE values. This spatial separation highlights the superior ability of ensemble-based methods like Random Forest to capture complex nonlinear relationships within multivariate experimental data. Figure 7B further supports these observations by comparing the actual versus predicted F_0/F values using the Quadratic Regression model. The blue scatter points represent individual predictions, while the red dashed line ($y = x$) denotes perfect agreement. Although the predictions generally follow the reference line, noticeable deviations are observed for certain data points, confirming the limitations of the quadratic model in fully capturing the response variability. These deviations may stem from unmodeled interactions or nonlinearities beyond second-order terms. Collectively, these results demonstrate that while the quadratic model offers interpretability and compatibility with RSM frameworks, the Random Forest model provides enhanced predictive power and generalization capability. The integration of machine learning approaches alongside statistical modeling can thus improve the reliability and precision of sensor-based analytical systems.

Figure 7

3.9. Calibration and Detection of Metronidazole

Under optimized conditions, the CQDs-based sensor exhibited a systematic fluorescence response to amoxicillin concentration (**Fig. 8**). Two linear working ranges were established to accommodate the dynamic behavior of the system: 10–300 μM (**Fig. 8A**) and 300–650 μM (**Fig. 8**). In each range, F_0/F was plotted against amoxicillin concentration, yielding highly coherent calibration lines suitable for quantitative analysis. The analytical sensitivity was quantified using the standard deviation of ten blanks and the corresponding slopes of the calibration lines, resulting in LODs of 41.47 μM and 101.78 μM and LOQs of 125.66 μM and 308.43 μM for the 10–300 μM and 300–650 μM ranges, respectively. These figures of merit indicate reliable detectability across low-to-moderate concentrations, with the first range offering superior sensitivity due to its steeper slope. The consistent blank precision (RSD = 3.39%) further supports the robustness of the measurements and the reproducibility of the sensing platform.

Collectively, the dual-range calibration enables accurate quantification of amoxicillin over a wide concentration window and provides a solid foundation for application in aqueous samples and routine analysis.

Figure 8

3.10. Interference Study for Amoxicillin Detection

To evaluate the selectivity of the CQDs-based sensor toward amoxicillin, a systematic interference study was conducted. The fluorescence quenching response (F_0/F) of 300 μM amoxicillin was measured alone and in the presence of 500 μM of various potentially interfering substances, including common ions and biomolecules: Ca^{2+} , K^+ , Na^+ , lactose, casein, vitamin C, riboflavin, triglycerides, and lactic acid. As shown in Fig. 4B, the F_0/F value for amoxicillin alone was 1.69, while the presence of interfering species resulted in only minor deviations. The most pronounced change was observed with Ca^{2+} (1.729) and triglycerides (1.721), followed by **vitamin C** (1.709) and riboflavin (1.702). However, these variations remained within acceptable analytical limits, with relative errors below 5%, indicating negligible impact on sensor performance. Other species such as K^+ (1.668), Na^+ (1.664), lactose (1.653), and casein (1.699) showed minimal interference, further confirming the sensor's robustness. These results demonstrate that the CQDs-based sensor exhibits high selectivity toward amoxicillin, even in the presence of structurally or chemically similar compounds. The minimal cross-reactivity highlights the potential of the system for reliable detection in complex biological or food matrices, where such interferents are commonly present.

3.11. Application

To evaluate the practical applicability of the developed CQDs-based fluorescent sensor, traditionally sourced dairy products were selected as representative real samples due to their relevance in food safety monitoring. The samples were homogenized and diluted tenfold in buffer solution (pH 7) to minimize matrix effects and simulate realistic analytical conditions. Under optimized sensing parameters, the sensor's performance was assessed by spiking known concentrations of amoxicillin (20, 300, and 600 μM) into the prepared dairy matrices. Fluorescence measurements were subsequently recorded, and the concentrations of amoxicillin were calculated using the established calibration model. The experimental results, summarized in Table 6, demonstrated excellent recovery values of 98.9%, 101.0%, and 100.1% for the respective spiked concentrations. Precision was also confirmed, with relative standard deviations (RSD%) of 3.14%, 1.08%, and 0.55%, respectively. These findings highlight the robustness of the CQDs-based sensor, showing that it maintains high accuracy and reproducibility even in complex food matrices. Thus, the proposed fluorescence method offers a rapid, sensitive, and eco-friendly approach for the quantification of amoxicillin in traditional dairy products, underscoring its potential for routine monitoring in food safety applications. A comparative overview of reported quantum dot-based probes for amoxicillin detection highlights the diversity of sensing strategies and their analytical performances. As summarized in Table 7, the CDs@CdTe@MIP ratiometric nanosensor exhibited a narrow linear range (0.1–50 μM) with a very low detection limit of 0.15 $\mu\text{g/L}$, and was successfully applied to spiked pork samples [11]. Similarly, the MIP/CdTe QDs-decorated polymer optic

fiber microprobe achieved an LOD of 0.31 $\mu\text{g/L}$ within a comparable range (0.5–50 $\mu\text{g/L}$), demonstrating applicability in both biological and environmental matrices [12]. The boron-doped carbon quantum dots extended the linear range significantly (1.43–429 μM) with an LOD of 0.825 μM , and were validated in milk and honey samples [13]. In contrast, this work employing CQDs-based sensors established two broad linear ranges (10–300 μM and 300–650 μM), with detection limits of 41.47 μM and 101.78 μM , respectively. Although the LOD values are higher compared to previously reported systems, the sensor demonstrates strong reproducibility, excellent recovery, and robust applicability in complex dairy matrices. This broader dynamic range is particularly advantageous for monitoring higher antibiotic residues in real food samples, where narrow-range sensors may fail to provide accurate quantification. Collectively, the comparison underscores that while earlier probes excelled in ultralow detection limits, the CQDs-based sensor developed herein offers a unique balance of wide linear range, matrix tolerance, and practical applicability, positioning it as a reliable platform for routine amoxicillin monitoring in food safety applications.

Table 6

Determination of tetracycline concentration in real samples. By fluorescence method (n = 5)

sample	Spiked value tetracycline (μM)	Found value by the sensor tetracycline (μM)	RSD%	Recovery (%)
1	20	19.78 $\pm$ 0.622	3.14	98.9
2	300	302.86 $\pm$ 3.27	1.08	101.0
3	600	600.62 $\pm$ 3.33	0.55	100.1
Relative standard error: RSD				

Table 7

Comparison of the performances of various sensors for detection of amoxicillin

Probe	Linear Range	LOD	Target	Real Sample	Ref
CDs@CdTe@MIP ratiometric nanosensor	0.1–50 μM	0.15 $\mu\text{g/L}$	Amoxicillin	Pork (spiked)	11
MIP/CdTe QDs-decorated polymer optic fiber microprobe	0.5–50 $\mu\text{g/L}$	0.31 $\mu\text{g/L}$	Amoxicillin	Biological & environmental matrices	12
boron-doped carbon quantum dots	1.43–429 μM	0.825 μM	Amoxicillin	Milk and honey	13
CQDs-based sensor	10–300 μM and 300–650 μM	41.47 μM / 101.78 μM	Amoxicillin	Dairy matrices (milk)	This work

Table 6

Table 7

Conclusions

In this study, a green, cost-effective, and versatile fluorescence-based sensor was successfully developed using carbon quantum dots (CQDs) synthesized via a hydrothermal route. Comprehensive structural and optical characterization by TEM, SEM, FTIR, XRD, EDX, mapping, and fluorescence spectroscopy confirmed the nanoscale morphology and functional surface groups of the CQDs. The sensor demonstrated effective detection of amoxicillin through a fluorescence quenching mechanism, exhibiting two broad linear ranges (10–300 μM and 300–650 μM) with satisfactory detection limits (41.47 μM and 101.78 μM) and excellent reproducibility ($\text{RSD} < 3.5\%$). Optimization of experimental parameters using Design of Experiments (DoE), response surface methodology (RSM), and central composite design (CCD) significantly enhanced analytical performance and provided insight into the influence of pH, reaction time, temperature, and sensor dosage. Furthermore, the integration of machine learning models (Random Forest vs. quadratic regression) improved predictive accuracy and highlighted the robustness of ensemble learning for complex multivariate data. Selectivity studies confirmed that common interferents—including metal ions, proteins, sugars, vitamins, and organic acids—did not significantly affect the fluorescence response, underscoring the sensor’s reliability in complex matrices. Application to real dairy samples demonstrated high recovery values ($\approx 99\text{--}101\%$) and strong precision, validating the practical utility of the system without extensive pretreatment. so, the developed CQDs-based nanoprobe provides a sensitive, selective, eco-friendly, and scalable platform for antibiotic monitoring. Its combination of structural characterization, DOE-based optimization, and machine learning evaluation positions it as a promising tool for food safety assessment and public health surveillance, particularly in traditional and artisanal dairy products.

Declarations

Acknowledgements

The authors would like to thank Islamic Azad University of Sanandaj for providing laboratory facilities and support.

Authors' contributions

Diba Adamia, Darya Barbod and Sara Bijari : Performed the experimental work, collected data, and contributed to the initial draft of the manuscript.

Changiz Karami: Conceived and designed the study, supervised the project, analyzed the data, and revised the manuscript critically.

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Ethics declarations

All milk samples used in this study were commercially available and purchased from local dairy sources in Kermanshah. The samples were used solely for analytical purposes, and no human or animal subjects were directly involved. Therefore, ethical approval and informed consent were not required for this study.

Consent to participate / Consent for publication

Not applicable.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Prescott JF. Beta-lactam antibiotics: penam penicillins. *Antimicrob therapy veterinary Med.* 2013;;133–52.
2. Belmar-Liberato R, Gonzalez-Canga A, Tamame-Martin P, Escribano-Salazar M. Amoxicillin and amoxicillin-clavulanic acid resistance in veterinary medicine—the situation in Europe: a review. *Vet Med.* 2011;56:473.
3. Costa LV, Gebara C, Zacaroni O, de Freitas F, Silva NE, Prado AN et al. CS,. Antibiotic Residues in Raw Cow's Milk: A Systematic Review of the Last Decade. *Foods.* 2024;13:3758.
4. Londoño-Carmona J, Blandón-Escobar S, Montoya-Zuluaga J, Betancourt-Chaves P, Castillo-Moreno S, Arboleda-Múnera C, et al. Antibiotic residues and microbial contamination in pasteurized whole milk intended for human consumption. *Veterinary World.* 2024;17:720.
5. Star BG, Shahlaei M, Karami C. A novel fluorescent turn-on probe for hydrogen peroxide based on carbon dots. *J Mater Sci: Mater Electron.* 2021;32:5615–23.
6. Ye C, Zhao Y, Fan C, Zhang T, Yang F, Zhang Y et al. A novel fluorescent probe for sensitive detection copper in food samples and in vivo. *Bioorg Chem.* 2026;;109464.

7. Kumar M, Nandi A, Yadav RL, Das Gupta G, Sharma K. AI-Enhanced Prediction Tools and Sensor Integration in Advanced Analytical Chemistry Techniques. *Curr Anal Chem*. 2025.
8. Constantinescu G, Schulze M, Peitzsch M, Hofmockel T, Scholl UI, Williams TA, et al. Integration of artificial intelligence and plasma steroidomics with laboratory information management systems: application to primary aldosteronism. *Clin Chem Lab Med (CCLM)*. 2022;60:1929–37.
9. Javaheri S, Keshavarzi F, Karami C. Development of co-doped ZnS-CdS quantum dots based composite sensor for the detection of cefixime (CFM) and tetracycline (TET), and application in real samples from local dairies. *Sci Rep*. 2025;15:10717.
10. Das P, Ganguly S, Banerjee S, Das NC. Graphene based emergent nanolights: A short review on the synthesis, properties and application. *Res Chem Intermed*. 2019;45:3823–53.
11. Li C, Ma Y, Fan C, He C, Ma S. Highly sensitive and selective detection of amoxicillin using molecularly imprinted ratiometric fluorescent nanosensor based on quantum dots. *Microchim Acta*. 2024;191:525.
12. Liang J, Wu Q, Xiao R, Yu R, Chen H, Tang Y, et al. Molecularly imprinted polymer/CdTe quantum dots-decorated polymer optic fiber microprobes for Amoxicillin detection. *Front Bioeng Biotechnol*. 2025;13:1700654.
13. Zhang X, Ren Y, Ji Z, Fan J. Sensitive detection of amoxicillin in aqueous solution with novel fluorescent probes containing boron-doped carbon quantum dots. *J Mol Liq*. 2020;311:113278.

Figures

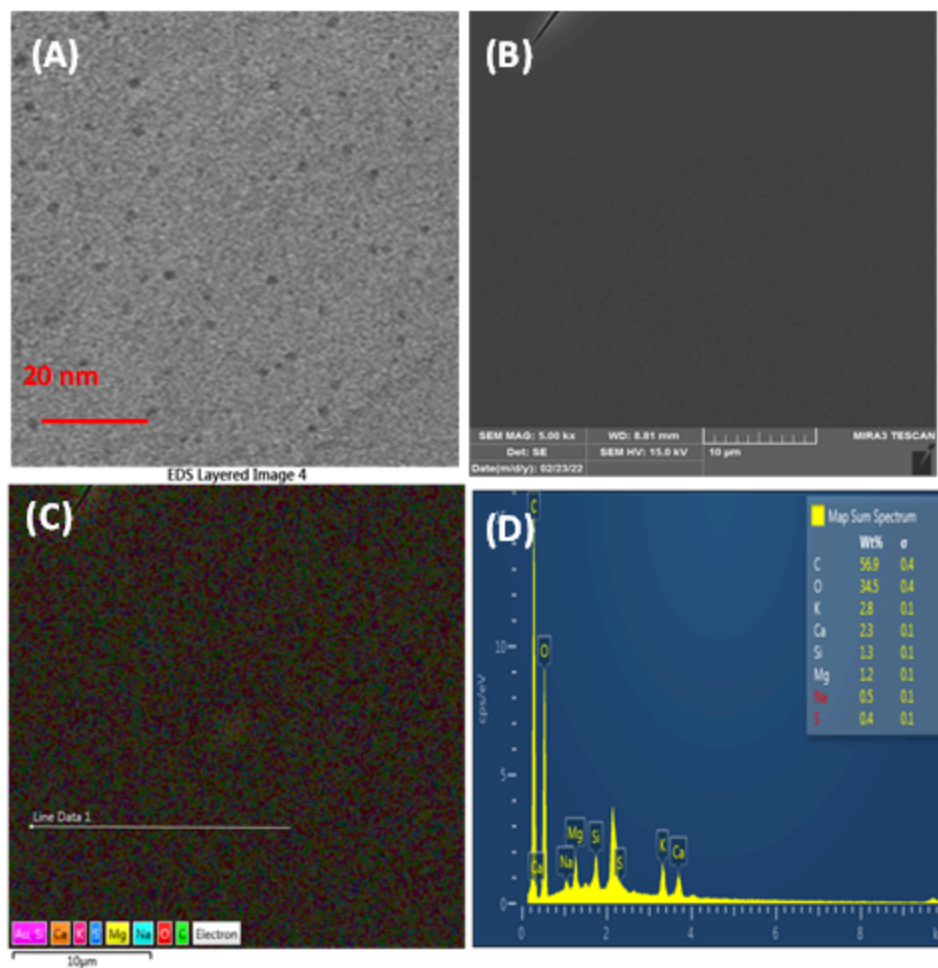


Figure 1

A) TEM images of CQDs SEM images, B) SEM imaging, C) mapping imaging C) EDS of CQDs

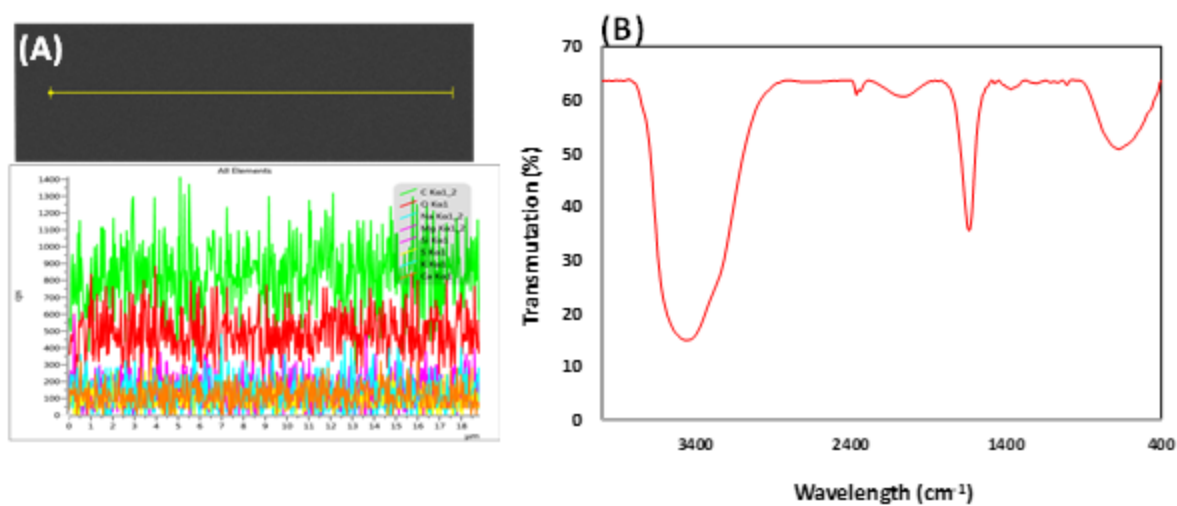


Figure 2

A) line scanning of CQDs, B) FTIR spectra of CQDs,

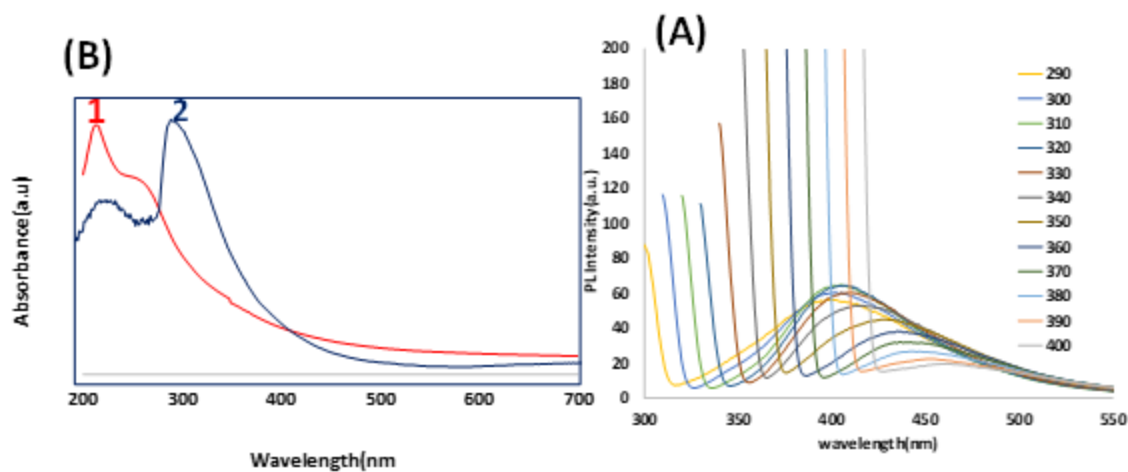


Figure 3

A) The UV/Vis absorption spectra revealed distinct optical characteristics between CQDs synthesized from pure glucose and those derived from *Quercus infectoria* extract **B)** Fluorescence spectrum of CQDs in excitation with different wavelengths from 280 nm to 350 nm

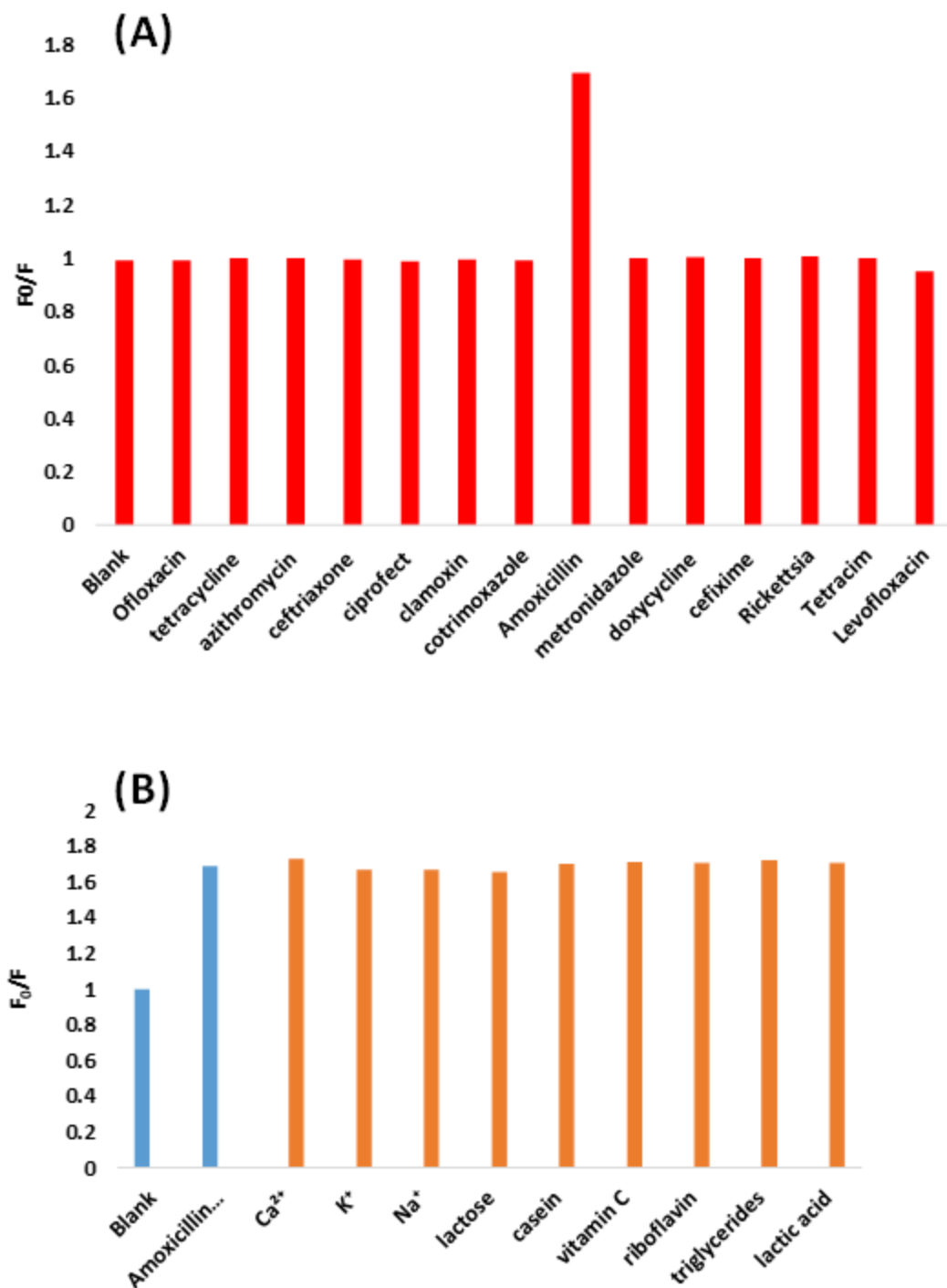


Figure 4

A) Fluorescence response of CQDs to various compounds, **B)** F_0/F ratio of CQDs in the presence of various compounds of blue rods represent amoxicillin alone, orange rods represent a mixture of amoxicillin (300 μ M) with other compounds (500 μ M).

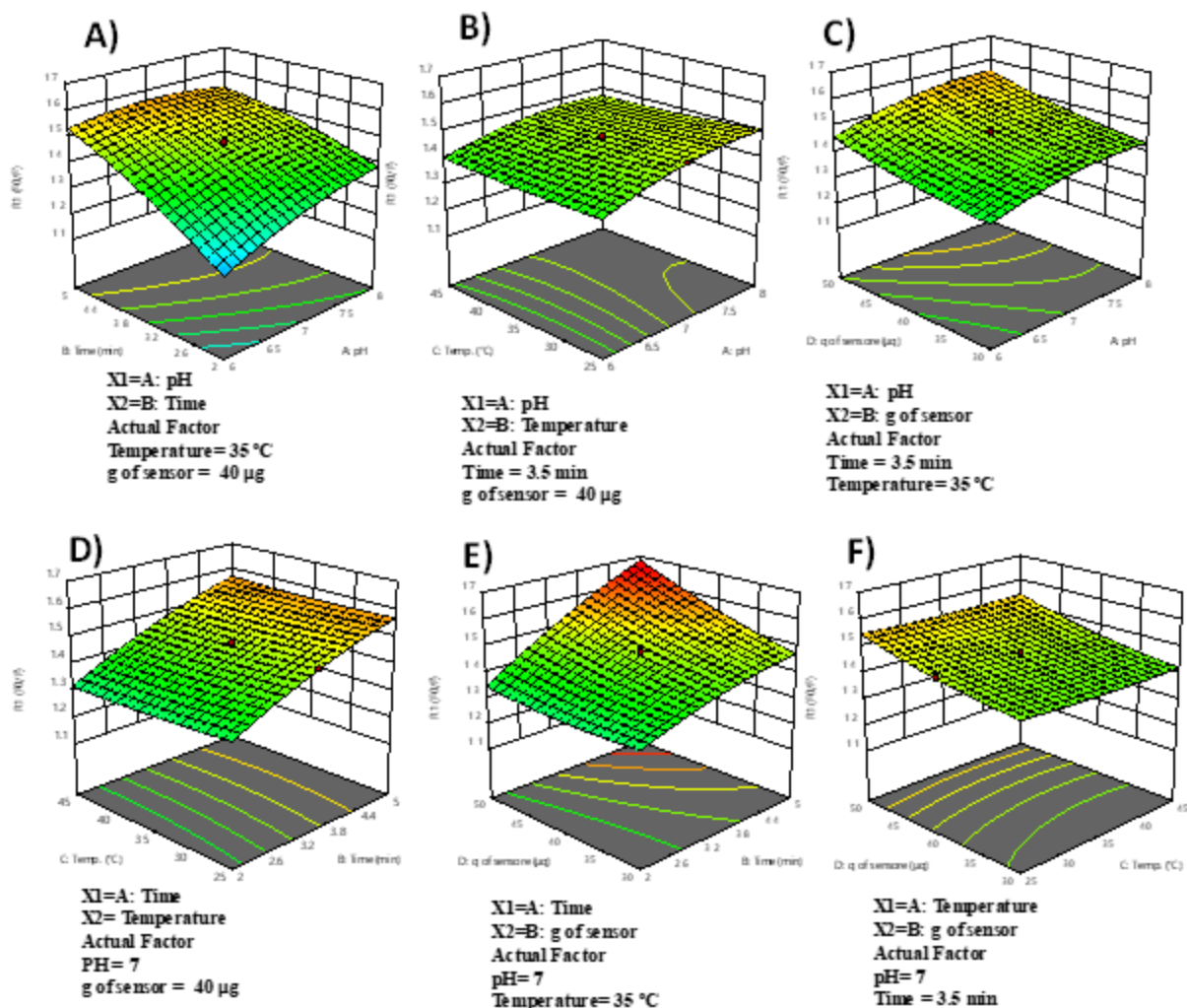


Figure 5

Three-dimensional response surface plots illustrating the interactive effects of experimental parameters on the fluorescence quenching efficiency (F_0/F) of the CQDs-based sensor for amoxicillin detection. (A) pH (A) vs. reaction time (B); (B) pH (A) vs. sensor dosage (D); (C) reaction time (B) vs. sensor dosage (D); (D) pH (A) vs. temperature (C); (E) reaction time (B) vs. temperature (C); (F) sensor dosage (D) vs. temperature (C)

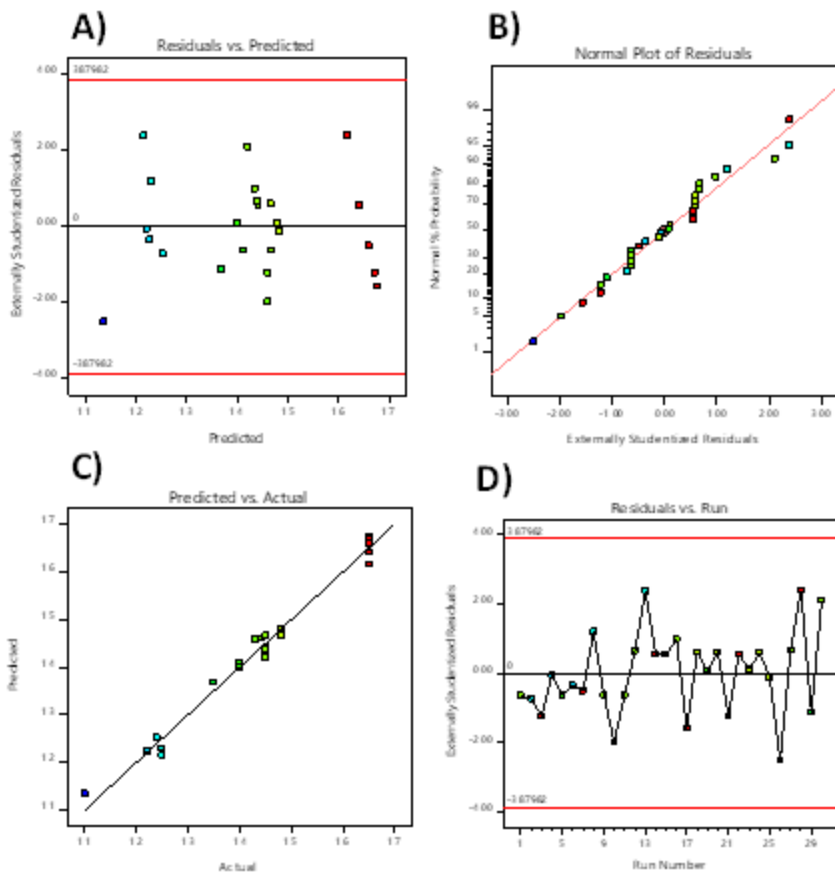


Figure 6

Diagnostic plots for the Central Composite Design (CCD) methodology adequacy (A) Residuals vs. Predicted plot (B) The Normal Plot of Residuals (C) residual versus fitted values and (D) Residuals vs. Run plot.

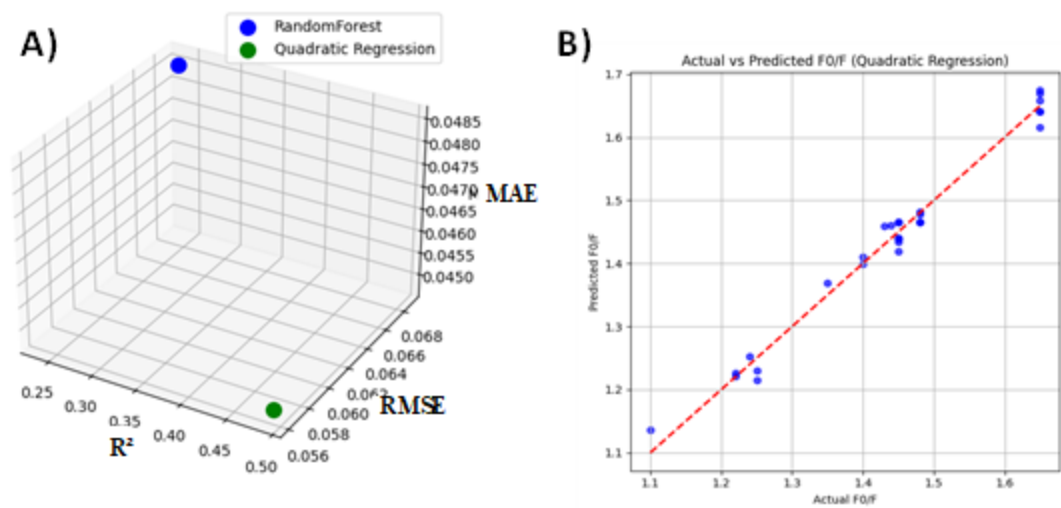


Figure 7

Comparison of regression models for amoxicillin detection. (A) Random Forest shows superior predictive accuracy (high R^2 , low RMSE/MAE) compared to Quadratic Regression. (B) Actual vs. predicted F_0/F using the quadratic model, with deviations from the $y = x$ line highlighting its limitations.

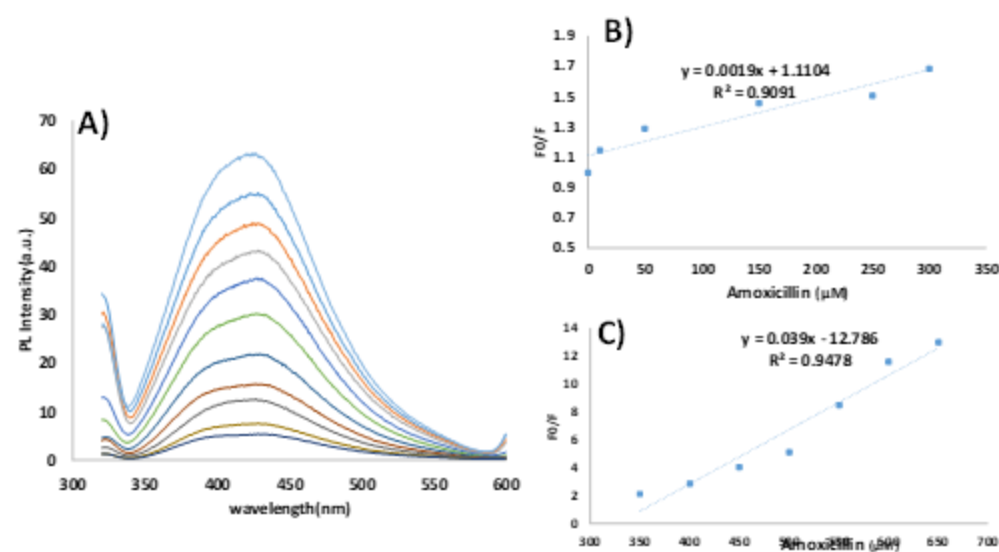


Figure 8

Spectral and analytical response of the CQDs-based sensor to amoxicillin. (A) Fluorescence emission spectra at varying amoxicillin concentrations, showing systematic quenching behavior. (B) Linear correlation between fluorescence intensity and amoxicillin concentration. (C) Absorbance–concentration plot confirming consistent optical response across the tested range. These results validate the sensor’s suitability for quantitative analysis via fluorescence and absorbance measurements.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryDataS1.xlsx](#)