

Sensitive Fluorescence Detection of Metronidazole Residues in Traditional Dairy Products Using Green-Synthesized Carbon Quantum Dots from *Rosa canina*: Combining Experimental Design and Machine Learning for Food Safety

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Abstract

In this study, CQDs were synthesized via a green hydrothermal method using *Rosa Canina* as a natural carbon source. The structural and optical features of the CQDs were analyzed using fluorescence spectroscopy, FTIR, SEM, EDX, and elemental mapping. The nanocomposite exhibited strong fluorescence emission, enabling sensitive detection of metronidazole (MNZ) through a fluorescence quenching mechanism. Sensor parameters were optimized with Design of Experiments (DoE), yielding a linear detection range of 5.0–400.0 μM , with a limit of detection of 2.24 μM and a limit of quantification of 7.39 μM . The sensor showed good repeatability (RSD = 3.38%, $n = 12$) and reproducibility (RSD = 3.47%). Machine learning was employed to improve predictive accuracy and data interpretation. The practical applicability was confirmed by the successful detection of MNZ in real dairy samples, with minimal matrix interference and satisfactory recovery.

Introduction

Ensuring food safety and quality is fundamental to safeguarding public health and supporting optimal growth and development. Beyond maintaining strict hygiene protocols during production, processing, and storage, food must be free from hazardous contaminants—particularly antibiotic residues. In recent decades, the extensive application of antibiotics in agriculture and livestock farming for disease prevention and treatment has grown substantially. However, the overuse and misuse of these substances have resulted in the accumulation of antibiotic residues in food products, raising significant concerns due to their potential adverse effects on human health [1, 2].

The extensive application of antibiotics in livestock production, while effective in combating bacterial infections, has significantly contributed to the development of antibiotic-resistant bacterial strains. This growing resistance poses a critical challenge to public health by complicating treatment protocols, increasing healthcare costs, and undermining global health security. Furthermore, the persistence of antibiotic residues in animal-derived food products—such as meat, milk, and eggs—has been associated with allergic reactions and various other adverse health effects in humans. Consequently, the rigorous monitoring and regulation of antibiotic usage within agri-food systems is imperative to mitigate these risks and safeguard both human and environmental health [3].

In addition to fostering antimicrobial resistance, the excessive use of antibiotics poses a significant threat to the integrity of the human gut microbiome—a highly diverse and dynamic ecosystem of beneficial microorganisms essential for maintaining host health. The gut microbiota is intricately involved in key physiological processes, including digestion, nutrient absorption, vitamin biosynthesis, and immune modulation. Disruption of this microbial balance due to antibiotic overexposure can result in gastrointestinal disturbances such as diarrhea and inflammation, as well as heightened vulnerability to chronic diseases. Therefore, ensuring food safety through stringent regulation of antibiotic use in animal agriculture is imperative not only to safeguard public health but also to preserve the long-term effectiveness of antimicrobial therapies [4].

Metronidazole is a broad-spectrum antimicrobial agent extensively utilized in both human and veterinary medicine for the treatment of infections caused by anaerobic bacteria and protozoa. Its application in livestock farming has become common practice for both therapeutic and prophylactic purposes. However, improper or excessive use of metronidazole in food-producing animals raises concerns regarding the presence of its residues in consumable animal products such as meat and milk. The ingestion of such contaminated foods may pose significant health hazards to consumers, including hypersensitivity reactions, alteration of the gut microbiota, and the potential emergence of metronidazole-resistant microbial strains. These risks underscore the urgent need for rigorous surveillance and regulatory control of antibiotic usage in animal agriculture to ensure food safety and public health protection [5, 6].

Chronic dietary exposure to metronidazole residues has been associated with a range of toxicological effects, including gastrointestinal disturbances such as nausea and vomiting, neurotoxicity, and potential carcinogenicity, which remains a topic of ongoing scientific debate. Moreover, sustained low-level intake of antibiotic residues contributes to the proliferation of resistant microbial strains, thereby complicating the management of infectious diseases in human populations. These pressing concerns highlight the urgent need for sensitive, accurate, and rapid analytical techniques to detect metronidazole residues in food products. Implementing such monitoring systems is essential for ensuring regulatory compliance, protecting consumer health, and preserving the long-term efficacy of antimicrobial therapies [7, 8].

Conventional techniques for the detection of metronidazole—such as high-performance liquid chromatography (HPLC), electrochemical assays, UV–visible spectroscopy, and immunoassays—have demonstrated satisfactory accuracy and reliability. However, these methods often require expensive instrumentation, extensive sample preparation, and highly trained personnel, limiting their practicality for routine and large-scale screening. Moreover, their performance may be compromised by matrix interferences, particularly in complex biological or food samples, and their sensitivity at trace levels can be inadequate for stringent safety standards. In light of these limitations, there is a growing demand for the development of innovative, simple, and highly sensitive detection platforms. Fluorescence-based sensors, particularly when integrated with advanced data processing techniques such as machine learning, represent a promising alternative. These approaches offer rapid, cost-effective, and on-site detection capabilities, enabling real-time monitoring of metronidazole residues. The adoption of such technologies not only enhances food safety and consumer protection but also contributes to the rational management of antibiotic use within the agri-food sector [9–12].

Fluorescence-based detection methods have garnered significant attention due to their exceptional sensitivity, high precision, and rapid analytical capabilities. These techniques are inherently cost-effective, compatible with small sample volumes, and often eliminate the need for complex instrumentation or extensive sample preparation. Their rapid response time and potential for real-time (online) monitoring make them especially attractive for food safety applications. When coupled with fluorescent nanosensors, these systems are capable of detecting ultra-trace levels of metronidazole with remarkable accuracy and minimal background interference, even in complex sample matrices. Such

integration enhances the reliability and applicability of fluorescence-based platforms for routine screening in food quality control and public health monitoring. [13].

In recent years, carbon quantum dots (CQDs) synthesized from bio-based and waste-derived materials have attracted considerable interest owing to their environmentally benign nature, cost-effectiveness, and remarkable optical properties [14]. In the present study, mold was employed as an innovative carbon precursor for the synthesis of CQDs. This strategy not only adheres to the core principles of green chemistry but also significantly enhances the fluorescence performance of the resulting nanosensor, thereby enabling highly sensitive detection of metronidazole. Utilizing mold as a carbon source adds value to biological waste, offering a sustainable and low-cost route for the fabrication of high-performance, biocompatible fluorescent probes. This approach presents a promising avenue for the development of next-generation sensing platforms that are both eco-friendly and analytically robust [15, 16].

In this context, the present study introduces a green and sustainable approach for the synthesis of carbon quantum dots using *Rosa Canina*, a natural and readily available plant source. The as-prepared CQDs were employed to develop a fluorescence-based sensor for the sensitive and selective detection of metronidazole in traditional dairy matrices. To enhance analytical performance and ensure robust sensor optimization, Design of Experiments (DoE) was applied, while machine learning algorithms were utilized for advanced data analysis and predictive modeling. This integrated strategy not only aligns with the principles of green analytical chemistry but also addresses the urgent need for rapid, cost-effective, and accurate monitoring of antibiotic residues in food products. The study further demonstrates the practical applicability of the developed sensor through successful detection of MNZ in real dairy samples, highlighting its potential for food safety surveillance and public health protection.

Experimental

2.1. Reagents and materials

Rosa canina plant material was collected by local harvesters from the Zagros region in western Iran, where the plant grows naturally and abundantly. The dried plant was purchased from a certified herbal shop (attari) in Kermanshah [17]. As *Rosa canina* is a non-endangered, widely available species in Iran, its collection and use do not require formal ethical approval or special permits. The plant material was air-dried under shade conditions and ground into fine powder. An aqueous extract was prepared by dissolving a defined quantity of the dried material in distilled water and filtering the solution to remove insoluble components. All chemicals used in this study, including trichloroacetic acid, were purchased from Merck (Germany). Reagents including glucose, CaCl_2 , MgCl_2 , MnCl_2 , NaCl, KCl, and phosphate-buffered saline (PBS, pH 7.4), along with bovine serum albumin (BSA), were obtained from Merck. The study also employed a series of antibiotics, namely Levofloxacin, Cefixime, Doxycycline, Metronidazole, Tetracycline, Cotrimoxazole, Clamoxin, Ciprofloxacin, Ceftriaxone, Azithromycin, Ofloxacin, and Amoxicillin, all purchased from the same supplier. The pH of all prepared solutions was adjusted to 6

using 0.1 M solutions of sodium hydroxide, hydrochloric acid, phosphoric acid, and sodium phosphate. All solutions were freshly prepared with double-distilled water to ensure reproducibility and purity.

2.2 Synthesis of Carbon Quantum Dots (CQDs)

Carbon quantum dots (CQDs) were synthesized using *Rosa Canina* mold derived from naturally fermented sour grape juice, which developed after three months of ambient storage. The mold biomass was carefully harvested, dried at 60 °C, and subsequently carbonized at 200 °C for 2 hours in a muffle furnace to yield a black carbonaceous powder. This powder was dispersed in deionized water and subjected to ultrasonic treatment at 40 kHz for 30 minutes to facilitate nanoparticle release. The resulting suspension was then filtered through a 0.22 µm membrane to isolate CQDs exhibiting strong fluorescence characteristics.

2.3 Characterization Techniques

The structural and morphological characteristics of the synthesized CQDs were comprehensively analyzed using various advanced techniques. Fluorescence emission spectra were obtained with a PerkinElmer LS 45 spectrofluorometer, while UV-Vis absorption spectra were recorded using a Cary 100 UV-Vis spectrophotometer. The presence of functional groups was confirmed through Fourier-transform infrared spectroscopy (FTIR) using a Thermo Avatar instrument. Surface morphology and elemental composition were examined via field emission scanning electron microscopy (FE-SEM, TESCAN MIRA III, 15 kV), complemented by energy-dispersive X-ray spectroscopy (EDX), elemental mapping, and line scan analysis.

2.4 Experimental Design and Optimization

To optimize the critical parameters influencing the performance of the CQDs sensor, a Central Composite Design (CCD) approach was applied using Design-Expert software (version 11.1.1.0, USA). Three independent variables—pH (4.0–10.0), reaction time (0.5–5.0 min), and temperature (34–60 °C)—were evaluated through 20 experimental runs, including factorial, axial, and six center points, as summarized in Table 1. A second-order polynomial model (Equation 1) was used to describe the relationship between the fluorescence response and the three factors, incorporating linear, quadratic, and interaction terms. The statistical significance of the model was confirmed by Analysis of Variance (ANOVA) with a 95% confidence level ($p < 0.05$). Furthermore, 3D response surface plots were generated to visualize the interactions and identify optimal sensor conditions.

$$Y = \beta_0 + (\beta_1 \times A) + (\beta_2 \times B) + (\beta_3 \times C) + (\beta_{11} \times A^2) + (\beta_{22} \times B^2) + (\beta_{33} \times C^2) + (\beta_{12} \times AB) + (\beta_{13} \times AC) + (\beta_{23} \times BC) \quad (1)$$

In the provided equation, Y represents the predicted response, and β_0 denotes the model constant. The coefficients β_1 , β_2 , β_3 , β_{11} , β_{22} , β_{33} , β_{12} , β_{13} , and β_{23} within the statistical model, the linear, quadratic, and interaction effects of factors A (pH), B (Temperature), and C (Time) on the response are demonstrated

Table 1

2.5 Fluorescence Sensing Procedure

A standardized fluorescence assay was established to evaluate the sensitivity of the synthesized carbon quantum dots (CQDs) toward varying concentrations of metronidazole (MNZ). In this procedure, 30 µg of CQDs was dispersed in 3 mL of aqueous solution buffered to pH 7.0. Fluorescence measurements were performed with an excitation wavelength of 420 nm, and emission spectra were recorded with a maximum emission centered at 550 nm. To assess the fluorescence response, measurements were carried out both in the absence (F_0) and presence (F) of metronidazole. The fluorescence quenching efficiency was quantified by calculating the F_0/F ratio, which served as a function of metronidazole concentration. This ratio provided a reliable metric for establishing a calibration curve and evaluating the analytical performance of the CQDs probe. The limit of detection (LOD) was calculated based on the signal-to-noise ratio (S/N) criterion of 3, following standard analytical chemistry protocols. This determination ensured the identification of the lowest concentration of MNZ that could be accurately and reproducibly detected by the developed fluorescence-based sensing system.

2.6 Machine Learning Analysis

To enhance the predictive accuracy and robustness of the fluorescence-based sensing system, various supervised machine learning algorithms were applied using Python (version 3.13.2) and the Scikit-learn library. A suite of regression models—including support vector regression (SVR), random forest regression (RFR), artificial neural networks (ANN), k-nearest neighbors (KNN), gradient boosting regression (GBR), and decision tree regression (DTR)—were trained and evaluated using the experimental dataset. The performance of each model was rigorously assessed based on statistical metrics such as the coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE). Among the tested algorithms, the model demonstrating the highest R^2 value and lowest error rates was selected as the optimal approach for accurate and reliable quantification of metronidazole.

2.7. Preparation of sample

To evaluate the practical applicability of the developed CQDs-based fluorescence sensor, raw milk samples were collected from local dairy sources via a nearby supermarket. Sample preparation followed a standardized protocol to ensure the effective extraction of analytes. Initially, 4 mL of raw milk was combined with 10 mL of distilled water in a 50 mL beaker, and 2 mL of 10% trichloroacetic acid was subsequently introduced to precipitate proteins. The mixture underwent ultrasonic treatment for 15 minutes at 25 °C, followed by centrifugation at 10,000 rpm for 10 minutes. The clear supernatant was then separated and neutralized with a 30% sodium hydroxide solution. followed by a second centrifugation at 20 °C for 10 minutes to remove residual particulates. For recovery assessments, selected milk samples were spiked with known concentrations of metronidazole (MNZ), processed under identical conditions, and analyzed using the optimized fluorescence sensing protocol [22].

Results and discussion

3.1 Structural and Morphological Characterization

To investigate the morphology and size of the synthesized carbon quantum dots (CQDs), **Fig. 1a** presents a detailed view of the surface morphology, providing insights into the particle shape as well as elemental composition within the quantum dots. The spherical morphology observed confirms the uniformity of the nanoparticles. Elemental mapping of the CQDs is depicted in **Fig. 1b**, highlighting the spatial distribution and relative abundance of elements on the nanoparticle surfaces. Carbon, represented in green, exhibits the highest density across the sample, indicating its dominant presence. Complementary to this, the energy-dispersive X-ray spectroscopy (EDX) spectrum shown in **Fig. 1c** quantitatively confirms the elemental composition, with carbon constituting approximately 50.9% and oxygen 26.0% the total elemental content. In addition to carbon, trace amounts of other elements such as potassium, calcium, magnesium, and sulfur were also detected within the CQDs structure. These elements are consistent with the plant-derived origin of the carbon source (*Rosa canina*), as these minerals are naturally present in plant tissues. Their presence further validates the green synthesis approach and may contribute to the physicochemical properties of the synthesized quantum dots.

transmission electron microscopy (TEM) was employed, as illustrated in **Fig. 2A**. The TEM analysis revealed that the CQDs possess a nearly spherical shape with an average diameter of approximately 2 nm. In **Fig. 2B**, the line scanning technique is utilized to visualize the spatial distribution of surface elements in the CQDs synthesized from *Rosa canina*. Each color denotes a specific element present in the sample—for instance, light green corresponds to carbon (C K α 1,2), red indicates oxygen (O K α 1), blue represents elements such as magnesium or potassium, while other colors signify the presence of additional detected elements. The variations in peak intensities along the scanned line demonstrate the heterogeneous distribution of these elements within the nanostructure. This element distribution highlights the incorporation of different elements both on the surface and internally in the CQDs, which can influence their optical, chemical, and biological properties.

Fig. 3A presents the FTIR spectra of the *Rosa canina* extract and the synthesized oak carbon quantum dots (R-CQDs), respectively. Both spectra exhibit characteristic absorption bands corresponding to similar functional groups, notably the carboxyl (–COOH) and hydroxyl (–OH) groups. These groups are identified by prominent peaks around 1700 cm⁻¹ and 3300 cm⁻¹, respectively, indicating the presence of these functionalities in both the precursor extract and the resulting quantum dots. The fluorescence characteristics of the synthesized carbon quantum dots (CQDs) were systematically examined under excitation wavelengths ranging from 280 nm to 350 nm. At excitation wavelengths between 280 nm and 320 nm, the emission spectra exhibited broad and diffuse peaks without distinct sharpness. However, as the excitation wavelength increased beyond 320 nm, the fluorescence peaks became noticeably sharper with significantly higher intensity. The most prominent emission was observed when the CQDs were excited at 360 nm, resulting in a well-defined emission peak centered at 450 nm. This excitation-emission pair (360 nm excitation and 450 nm emission) represents the optimal condition for fluorescence in the CQDs synthesized from *Rosa canina* (**Fig. 3B**).

The UV-Vis spectra display notable differences between glucose-derived CQDs (a) and Rosa canina-derived CQDs (b) **Fig. S1**. The glucose-derived CQDs exhibit a broad absorption peak around 200–300 nm, characteristic of π - π^* transitions of aromatic carbon structures. In contrast, the Rosa canina-derived CQDs show a distinct peak near 350 nm, indicating the presence of surface states associated with oxygen-containing functional groups. Elemental analysis reveals that both CQDs consist predominantly of carbon (approximately 50.9%) and oxygen (26.0%), with trace amounts of elements such as potassium, calcium, magnesium, and sulfur, which are integrated into the CQDs structure. These elements, along with the functional groups identified via spectroscopic analysis, suggest that Rosa canina-derived CQDs possess a higher degree of surface functionalization, likely due to phytochemicals from the plant source. This enhanced surface chemistry contributes to the more pronounced absorption features observed in the UV-Vis spectrum.

Fig. 1

Fig. 2

Fig. 3

3.2. Statistical analysis

In order to systematically evaluate and optimize the fluorescence quenching response (F_0/F) of the developed CQDs sensor for metronidazole detection, a Central Composite Design (CCD) based on Response Surface Methodology (RSM) was employed. Sixteen experimental runs were performed to explore the combined effects of pH (A), temperature (B), and reaction time (C) on sensor performance. The experimental conditions and corresponding F_0/F values are summarized in **Table 2**. The response values ranged from 1.09 to 1.16, indicating that changes in the studied parameters had a measurable influence on the fluorescence quenching behavior. Among the tested models (linear, two-factor interaction [2FI], and quadratic), the quadratic model demonstrated the best statistical performance. As shown in Table 3, this model exhibited a high adjusted R^2 of 0.9919 and a predicted R^2 of 0.9653, suggesting a strong correlation between experimental and predicted responses and minimal overfitting. The model's sequential p-value was < 0.0001 , confirming its statistical significance. Moreover, the relatively low lack-of-fit p-value (0.0005) further validates the model's adequacy and suitability for prediction. The final quadratic model equation, derived in terms of coded variables, is expressed as:

$$Y = 1.16 + 0.0012A + 0.0075B - 0.0000C - 0.0212A^2 - 0.0175B^2 - 0.0003C^2 - 0.0025AB + 0.0000AC + 0.0000BC$$

In this model, positive coefficients denote synergistic contributions to fluorescence intensity, while negative coefficients imply antagonistic or inhibitory effects. The dominant influence of temperature and pH, both individually and in interaction terms, highlights their critical roles in modulating the sensing behavior. Taken together, the statistical analysis confirms that the quadratic model provides a reliable

and predictive framework for optimizing operational parameters and achieving maximal sensor response.

Table 2

Table 3

3.3. ANOVA

To evaluate the statistical robustness of the quadratic model constructed for predicting the fluorescence quenching response (F_o/F), an Analysis of Variance (ANOVA) was conducted, and the results are presented in Table 4. The model demonstrated a highly significant overall fit, as evidenced by an F-value of 260.94 and an associated p-value < 0.0001, indicating that the probability of this model occurring due to random variation is less than 0.01%. Among the independent variables and interaction terms, reaction time (B) and the interaction between pH and time (AB) emerged as statistically significant contributors, with p-values of < 0.0001 and 0.0073, respectively. Additionally, the quadratic terms for pH (A^2) and time (B^2) showed extremely high significance ($p < 0.0001$), highlighting the non-linear effects of these variables on the sensor response. In contrast, variables such as temperature (C), its interaction terms (AC, BC), and its quadratic effect (C^2) exhibited p-values much greater than 0.05, indicating negligible influence under the tested conditions. These results suggest that while temperature is included for model completeness and to maintain hierarchy, it does not significantly affect the fluorescence response in the studied range. The lack-of-fit test yielded a p-value of 0.0005, suggesting a statistically significant deviation between model predictions and actual data. However, considering the minimal residual error (Mean Square Residual = 4.45E-6) and high model significance, this deviation is considered acceptable within the context of analytical variability. Overall, the ANOVA results confirm that the quadratic model is robust and reliable, with critical contributions from reaction time and pH, especially in their squared and interactive forms. This reinforces the utility of CCD-RSM in accurately modeling and optimizing complex sensing systems. This confirms the model’s predictive capability and suitability for response surface optimization. The final fitted quadratic equation, derived in terms of actual values of the variables, is presented below:

$$Y = +0.9830 + (0.0395 \times A) + (0.0131 \times B) - (0.0001 \times C) - (0.0033 \times A^2) - (0.0005 \times B^2) + (1.3567 \times C^2) - (0.0001 \times AB) + (0.0000 \times AC) + (0.0000 \times BC) \tag{2}$$

This equation illustrates how each factor contributes to the fluorescence intensity and allows for effective prediction and optimization of conditions for metronidazole analysis.

Table 4

3.4. 3D response surface plots

To explore the synergistic and individual impacts of the experimental variables—pH (A), Time (B), and Temperature (C)—on the fluorescence response (F_o/F), three-dimensional response surface plots were

generated based on the fitted quadratic model. Each plot illustrates the effect of two variables at a time, while the third variable is held at its central level, enabling a comprehensive understanding of interactive behaviors. **Fig. S2A** represents the relations between pH and Time. The pronounced curvature of the surface, along with concentric elliptical contour lines, signifies a significant interaction effect. The response initially increases with both pH and time, reaches a peak, and then declines, reflecting a non-linear dependency. This pattern is indicative of an optimum reaction window where fluorescence quenching is maximized, likely due to optimal molecular interactions or surface energy states of the sensing system. **Fig. S2B** illustrates the interaction between pH (A) and Temperature (C). While the surface still exhibits curvature, the contours are more linear and parallel, indicating a weaker interaction compared to the A-B pair. The fluorescence response shows moderate sensitivity to pH, but only slight variation with temperature, suggesting that pH is the dominant factor in this interaction, while temperature exhibits a mild dampening effect at higher pH levels, possibly due to reduced structural stability of the quantum dots or target analyte at elevated temperatures. **Fig. S2C** demonstrates the relationship between Time (B) and Temperature (C). Here, the response surface is relatively flatter with nearly straight, parallel contour lines, suggesting a minimal interactive effect between these two variables. The fluorescence intensity increases gradually with time but remains largely unaffected by temperature across the studied range. This indicates that reaction time has a more pronounced influence than temperature under the given experimental constraints. Collectively, the 3D response surfaces confirm that pH and Time are the most influential parameters governing the fluorescence quenching behavior, with Temperature playing a lesser role. The presence of distinct optima on the surfaces highlights the effectiveness of RSM in identifying precise experimental conditions for maximum sensing performance.

3.5. Accuracy of the Model

The statistical evaluation of the developed quadratic model for predicting the fluorescence quenching response (F_0/F) demonstrates excellent accuracy and reliability, as summarized in Table 5. The coefficient of determination (R^2) was calculated to be 0.9958, indicating that approximately 99.58% of the variability in the fluorescence response can be explained by the model. This high R^2 value reflects a strong correlation between the predicted and actual values. Furthermore, the adjusted R^2 value of 0.9919 confirms the model's robustness even after accounting for the degrees of freedom associated with the number of predictors, while the predicted R^2 of 0.9653 remains in close agreement with the adjusted R^2 . The small difference between these two indices (< 0.03) affirms the model's predictive reliability and generalizability to unseen data. The standard deviation (SD) of 0.0021 and a very low coefficient of variation (C.V.) of 0.1859% suggest minimal experimental error and high precision across the response data. Moreover, the adequate precision value was found to be 45.2147, far exceeding the acceptable threshold of 4. This substantial value indicates a strong signal-to-noise ratio and confirms that the model can effectively navigate and differentiate within the experimental design space. Taken together, these statistical indicators validate the accuracy, reproducibility, and predictive strength of the quadratic model, thereby supporting its application for the precise optimization of fluorescence-based metronidazole sensing using carbon quantum dots.

Table 5

3.6 Model Diagnostics and Assumptions Verification

To evaluate the adequacy and statistical soundness of the developed quadratic model, several diagnostic plots were analyzed (**Fig. S3A–D**). These plots help assess the fundamental assumptions of regression, including normality, homoscedasticity, and independence of residuals. In **Fig. S3A** (Residuals vs. Predicted Values), the residuals are randomly scattered around the horizontal axis without any clear pattern. This uniform spread indicates homoscedasticity, meaning the variance of residuals is consistent across the range of predicted values, satisfying a key assumption of regression. **Fig. S3B** (Normal Probability Plot of Residuals) demonstrates that the residuals follow a nearly straight line, suggesting they are approximately normally distributed. This supports the validity of the model and indicates the absence of significant skewness or outliers that might distort the predictions. In **Fig. S3C** (Predicted vs. Actual Values), most points lie close to the diagonal line, reflecting a high level of agreement between experimental and predicted values. This alignment confirms the strong predictive power of the model across the studied response range, though a slight deviation is observed at extreme values, which may suggest mild model over- or under-estimation in those regions. **Fig. S3D** (Residuals vs. Run Order) reveals no obvious trend or pattern, indicating that the residuals are independent over time and that no systematic error occurred during the experimental process. Overall, the diagnostic plots collectively confirm that the model meets the essential regression assumptions. The residuals exhibit normality, constant variance, and independence, reinforcing the reliability and robustness of the model for optimization and prediction in the amoxicillin removal process.

Fig.8

3.7. Machine Learning Model Evaluation for Metronidazole Prediction

To compare the predictive performance of different machine learning models for metronidazole detection, three widely used algorithms—Linear Regression, Random Forest, and Support Vector Regression (SVR)—were evaluated using statistical metrics: coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE). **Fig.4A** presents a three-dimensional performance comparison of the models in R^2 –RMSE–MAE space. The Random Forest model is clearly distinguished from the others, positioned in the optimal region characterized by the highest R^2 (~0.96) and the lowest RMSE and MAE values. This spatial separation highlights the Random Forest model’s superior ability to capture the underlying nonlinear relationships within the data, ensuring both accuracy and reliability in prediction. In contrast, both Linear Regression and SVR cluster in the region associated with poor performance, exhibiting negative R^2 values and substantially higher error metrics. This indicates their inability to effectively model the complexity of the dataset, likely due to oversimplified assumptions (linear and kernel-based, respectively) that fail to generalize across data variations. **Fig. 4B** further supports these observations by comparing the actual vs. predicted values for each model. The Random Forest predictions (green points) align closely with the ideal diagonal line, reflecting minimal deviation and high agreement with experimental values. Meanwhile, predictions from Linear Regression (red) and

SVR (blue) deviate noticeably from the reference line, confirming their relatively poor predictive accuracy. Collectively, these results underscore the advantages of ensemble-based machine learning models like Random Forest in handling complex, multivariate data typically encountered in analytical chemistry and sensor development. Their robustness, interpretability, and precision make them valuable tools for enhancing detection methodologies and data-driven optimization in real-world applications.

Fig.4

3.8. Method Selectivity

To evaluate the selectivity of the developed sensing system, various commonly used antibiotics and related compounds were tested, including amoxicillin, azithromycin, ceftriaxone, cefixime, ciprofloxacin, clamoxin, cotrimoxazole, doxycycline, levofloxacin, metronidazole, ofloxacin, rickettsia, tetracim, tetracycline, and others. Each compound was added to the sensing medium at equal concentrations, and the resulting fluorescence intensity changes were recorded and normalized against the blank sample. As shown in the data, the fluorescence response remained nearly constant (close to 1) for most tested compounds, indicating minimal or no interaction with the sensing material. However, a distinct increase in fluorescence intensity was observed in the presence of metronidazole, with a normalized value of 1.23, suggesting a significant interaction with the sensing platform. In contrast, doxycycline exhibited only a negligible change (1.002), and thus cannot be considered a significant interferent in this context. These results demonstrate that the developed system shows a selective fluorescence enhancement response toward metronidazole, while exhibiting minimal interference from other structurally or functionally similar compounds. This highlights the method's high selectivity for metronidazole detection in complex aqueous environments, making it a promising approach for selective antibiotic sensing (**Fig. S4**).

3.9. Calibration and Detection of Metronidazole

The synthesized CQDs derived from Rosa Canina exhibited a strong fluorescence emission, which was significantly modulated upon interaction with metronidazole (MNZ). Under optimized experimental conditions, the fluorescence intensity of the CQDs increased gradually with rising concentrations of MNZ, forming the foundation for their application as a sensitive and eco-friendly fluorescence-based sensor. To evaluate the analytical performance, various concentrations of MNZ ranging from 5.0 to 400.0 μM were introduced into the sensing system, and the ratio of fluorescence intensities in the absence and presence of MNZ (F_0/F) was plotted as a function of MNZ concentration. As illustrated in **Fig. 5A**, the sensor displayed a clear linear response within this concentration range, with a correlation coefficient (R^2) indicative of excellent linearity and precision. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve, yielding values of 2.24 μM and 7.39 μM , respectively (**Fig. 5B**). These results demonstrate the high sensitivity of the proposed sensor system. Notably, the fluorescence enhancement mechanism is attributed to the interaction between metronidazole and the surface functional groups of the CQDs, which may alter the local electronic environment and promote radiative recombination. The good

linearity, along with low detection limits, confirms the capability of the CQDs-based sensor to perform accurate and reliable quantification of metronidazole in aqueous samples.

Fig. 5

3.10. Interference Study for Metronidazole Detection

carried out by adding common coexisting compounds to the metronidazole solution. The fluorescence intensity of 250 μM metronidazole alone was compared to that obtained in the presence of 500 μM of each interfering substance (Ca^{2+} , K^+ , Na^+ , lactose, casein, vitamin C, riboflavin, triglycerides, lactic acid, and tetracycline). The results revealed that the fluorescence signal remained nearly unchanged when the interfering species were added, indicating that they did not significantly influence the detection of metronidazole. This suggests a high degree of specificity of the CQDs-based sensor even in complex sample matrices. Thus, the developed sensor can reliably quantify metronidazole without substantial interference from other typical food components (**Fig.S5**).

3.11. Application

To assess the practical applicability of the developed CQDs-based fluorescent nanoprobe, traditionally sourced dairy products were selected as representative real samples, due to their relevance in food safety monitoring. The dairy samples underwent an initial preparation involving homogenization and dilution (10-fold) in a buffer solution adjusted to pH 5, to reduce matrix complexity and mimic real analytical conditions. Under optimized conditions, the nanoprobe's performance was evaluated by spiking known concentrations of metronidazole (10, 150, and 300 μM) into the prepared dairy matrices. Subsequent fluorescence measurements were recorded, and the concentration of metronidazole was calculated based on the established calibration model. The experimental data, summarized in **Table 6**, revealed excellent recovery values of 100.5%, 101.7%, and 100.63%, for the respective spiked concentrations. Additionally, the method demonstrated strong precision, with relative standard deviations (RSD%) of 2.94%, 2.54%, and 1.12%, respectively. These results confirm that the developed CQDs sensor possesses high reliability, precision, and accuracy, even in complex food matrices. Thus, the proposed fluorescence-based method offers a rapid, sensitive, and environmentally friendly approach for the quantification of metronidazole in traditional dairy products, highlighting its potential for routine monitoring in food safety applications. **Table 7** summarizes the analytical performances of several sensors reported for metronidazole detection, highlighting their linear ranges, limits of detection (LOD), and application in real sample matrices. Sodium-based carbon quantum dots demonstrated a linear range of 20–100 μM with an LOD of 62.5 nM, applied to water samples. The molecularly imprinted polymer sensor exhibited a linear range of 5.0–60.0 μM and an LOD of 1.28 μM , tested in real samples. A nitrogen-doped fluorescent carbon dots sensor provided a more sensitive detection with a linear range of 0.5–22 μM and an LOD of 0.22 μM in urine samples. The CQDs sensor developed in this work shows a competitive linear range from 5.0 to 350.0 μM with an LOD of 2.24 μM , successfully applied to serum samples. Although its LOD is higher compared to some previously reported sensors, the present probe

benefits from a wider linear range and demonstrated practical applicability in complex biological matrices, supporting its potential for clinical metronidazole monitoring [18–20].

Table 6

Table 7

Conclusions

In this study, a green, cost-effective, and highly sensitive fluorescence-based sensor was developed using carbon quantum dots (CQDs) synthesized from Rosa Canina via a hydrothermal method. The CQDs demonstrated excellent structural and optical characteristics, confirmed by TEM, FTIR, SEM, EDX, Iain Scan, mapping and fluorescence analyses. The sensor effectively detected metronidazole (MNZ) through a fluorescence quenching mechanism, exhibiting a wide linear detection range (5.0–350.0 μM), a low limit of detection (2.24 μM), and high repeatability and reproducibility. Optimization using Design of Experiments (DoE) significantly improved analytical performance. Moreover, the integration of machine learning algorithms enhanced predictive modeling and provided deeper insights into the sensor's behavior under varying conditions. Selectivity studies confirmed that the presence of common interferents—including metal ions, proteins, sugars, and structurally similar compounds—did not significantly impact the fluorescence response. The method also demonstrated high recovery rates in real dairy matrices, validating its applicability in complex food samples without extensive pretreatment. Overall, the developed CQDs-based nanoprobe offers a reliable, eco-friendly, and scalable platform for antibiotic monitoring. Its high sensitivity, selectivity, and compatibility with real samples position it as a promising tool for food safety assessment and public health surveillance, particularly in traditional and artisanal dairy products.

Declarations

Acknowledgements

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Author contributions

Sepideh Gharehyakheh: Conducted the experimental work, synthesized the carbon quantum dots, and collected fluorescence data. Changiz Karami: Assisted in data analysis, machine learning modeling, and optimization experiments. Sadaf Pirouzi: Contributed to study design, manuscript preparation, and data interpretation. All authors reviewed and approved the final version of the manuscript. Sepideh Gharehyakheh is the corresponding author. **Competing interests** Kimiya Khandestana, Azar Sabukbara, Bahareh Rahimian Zarif, Nahid Haghnazari, and Nasser Harzandi declare no competing interests.

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.

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Tables

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	5.50	3.00	8.00	0.0000	Actual
B	Time	9.50	4.00	15.00	0.0000	Actual
C	Temp.	45.00	30.00	60.00	0.0000	Actual

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	5.5	9.5	70	1.16
2	3	4	60	1.11
3	5.5	0.5	45	1.1
4	5.5	9.5	45	1.16
5	5.5	9.5	45	1.16
6	5.5	9.5	45	1.16
7	5.5	9.5	45	1.16
8	5.5	19	45	1.12
9	8	4	30	1.12
10	8	4	60	1.12
11	3	15	60	1.13
12	5.5	9.5	20	1.16
13	10	9.5	45	1.09
14	2	9.5	45	1.12
15	3	15	30	1.13
16	5.5	9.5	45	1.16
17	5.5	9.5	45	1.16
18	8	15	60	1.13
19	3	4	30	1.11
20	8	15	30	1.13

Table 3: Model summary statistic.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.7551		-0.1047	-0.4540	
2FI	0.9953		-0.3527	-1.2849	
Quadratic	< 0.0001		0.9919	0.9653	Suggested
Cubic			1.0000		Aliased

Table 4: ANOVA for response surface quadratic model for F₀/F

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0105	9	0.0012	260.94	< 0.0001	significant
A-pH	0.0000	1	0.0000	3.86	0.0779	
B-Time	0.0008	1	0.0008	172.62	< 0.0001	
C-Temp.	1.735E-18	1	1.735E-18	3.898E-13	1.0000	
AB	0.0000	1	0.0000	11.24	0.0073	
AC	1.735E-18	1	1.735E-18	3.898E-13	1.0000	
BC	1.735E-18	1	1.735E-18	3.898E-13	1.0000	
A ²	0.0058	1	0.0058	1302.56	< 0.0001	
B ²	0.0044	1	0.0044	997.08	< 0.0001	
C ²	1.310E-06	1	1.310E-06	0.2944	0.5993	
Residual	0.0000	10	4.450E-06			
Lack of Fit	0.0000	5	8.900E-06			
Pure Error	0.0000	5	0.0000			
Cor Total	0.0105	19				

Table 5: Standard deviation and R² of the response.

Std. Dev.	0.0021	R²	0.9958
Mean	1.13	Adjusted R²	0.9919
C.V. %	0.1859	Predicted R²	0.9653
		Adeq Precision	45.2147

Table 6: Determination of metronidazole concentration in real samples. By fluorescence method (n = 5)

sample	Spiked value metronidazole (μM)	Found value by the sensor metronidazole (μM)	RSD%	Recovery (%)
1	10	10.5±0.27	2.94	100.5
2	150	151.60±3.85	2.54	101.7
3	300	352.20±3.96	1.12	100.63

Relative standard error: RSD

Table 7: Comparison of the performances of various sensors for detection of metronidazole

Probe	Linear range	LOD	Antibiotics	Real sample	[Ref]
sodium based-carbon quantum dots	20–100 μM	62.5 nM	metronidazole	water samples	[18]
molecular imprint polymer for metronidazole extraction–detection	5.0-60.0 μM	1.28 μM	metronidazole	real samples	[19]
nitrogen-doped fluorescent carbon dots	0.5–22 μM	0.22 μM	metronidazole	urine samples	[20]
CQDs of mod	5.0-350.0 μM	2.24 μM	metronidazole	serum samples	This work

Figures

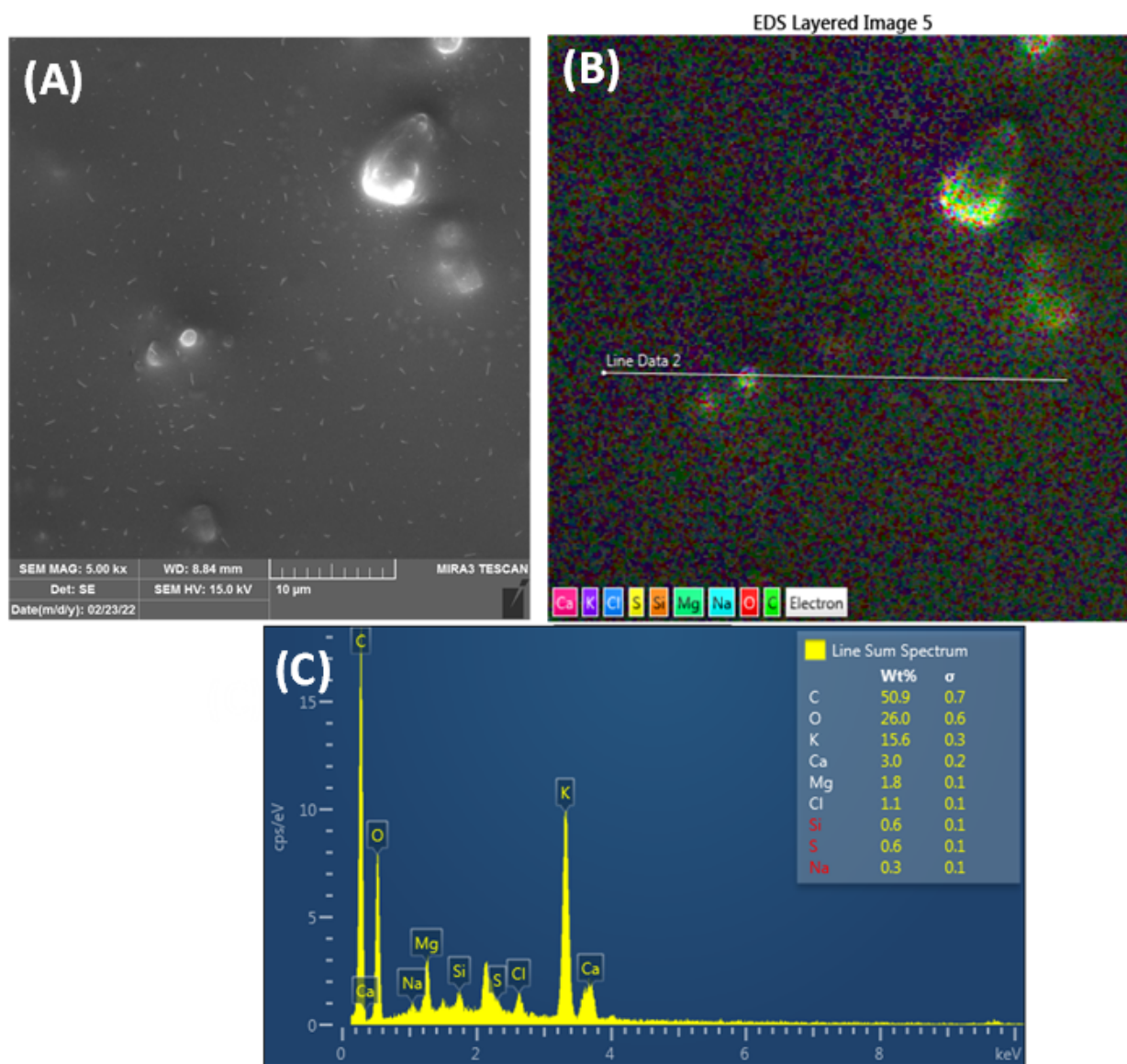


Fig. 1

Figure 1

A) SEM images, mapping imaging, C) EDS of R-CQDs

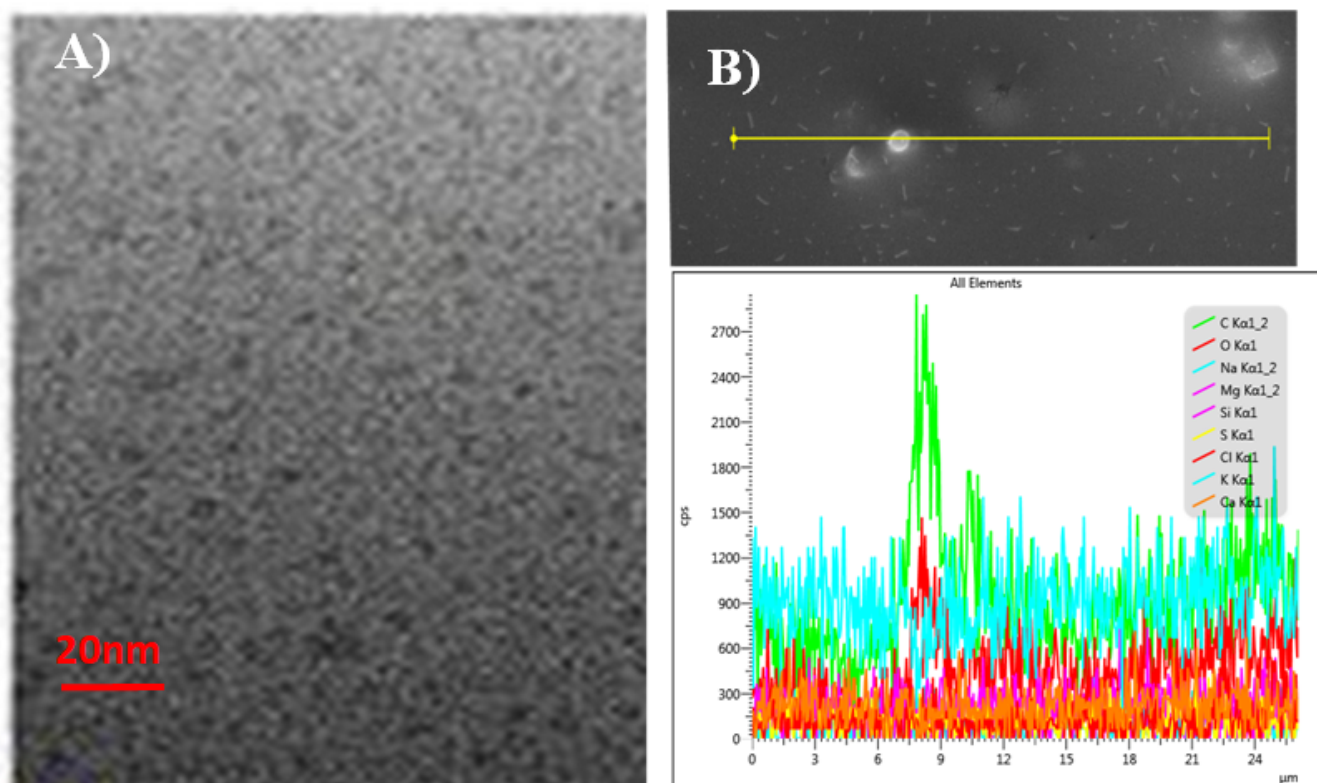


Fig. 2

Figure 2

A) TEM images of R-CQDs, B) line scanning of R-CQDs

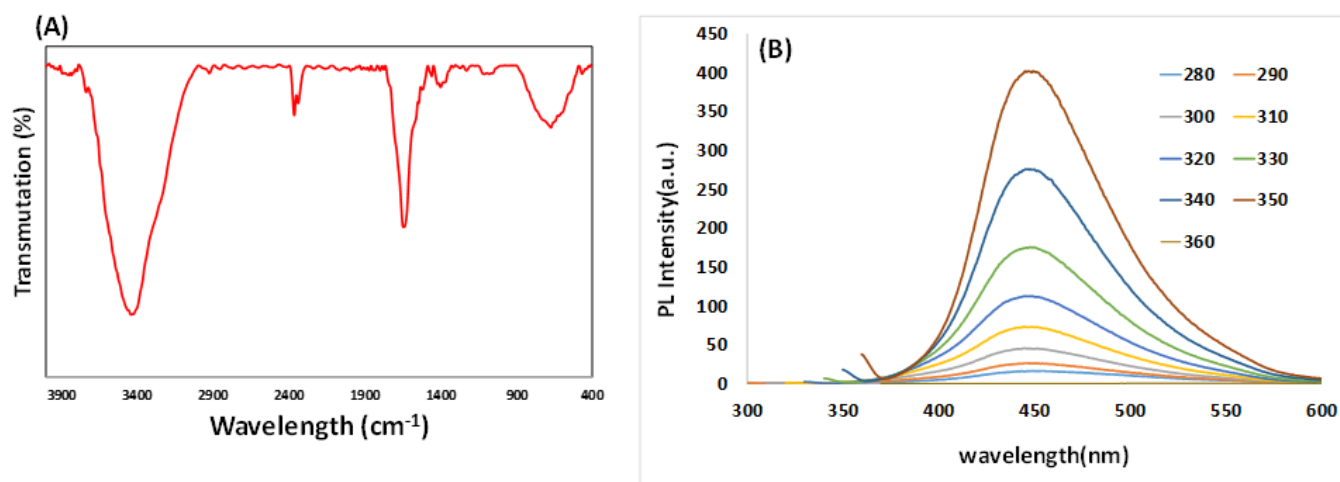


Fig.3

Figure 3

A) FTIR spectra of R-CQDs, B) Fluorescence spectrum of R-CQDs in excitation with different wavelengths from 280 nm to 350 nm

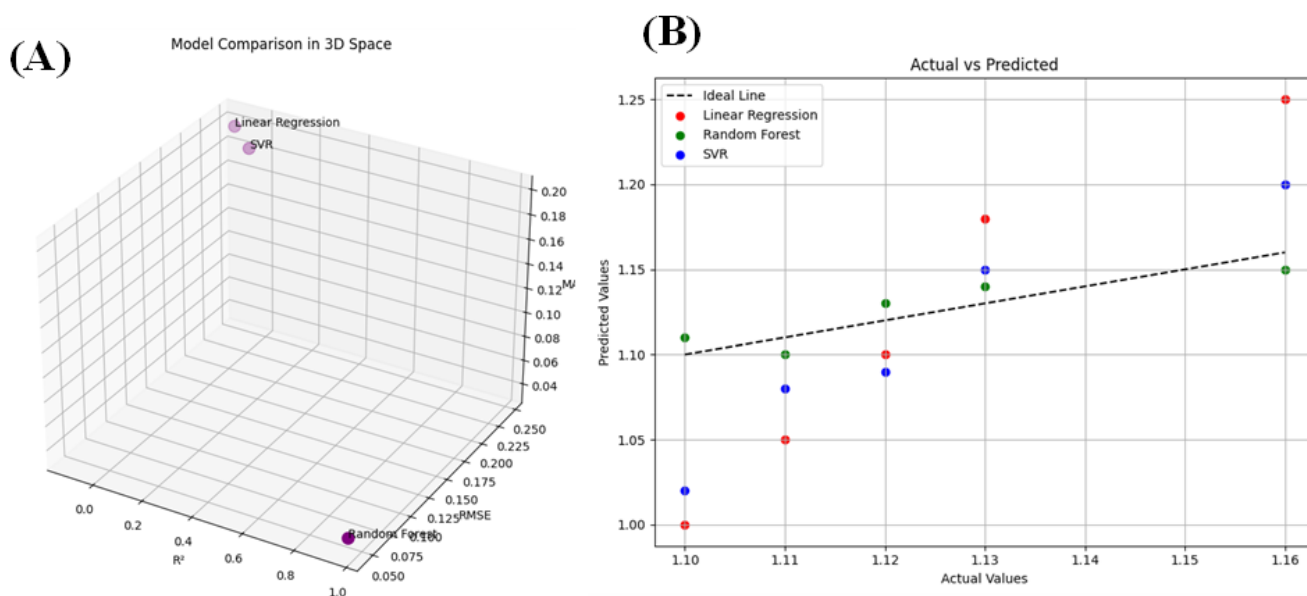


Fig.4

Figure 4

(A) 3D Scatter Plot of Model Performance. The plot visualizes the Mean Squared Error (MSE) and R-squared values for each model, with the z-axis representing the model indices, (B) Model Performance: Actual vs. Predicted values

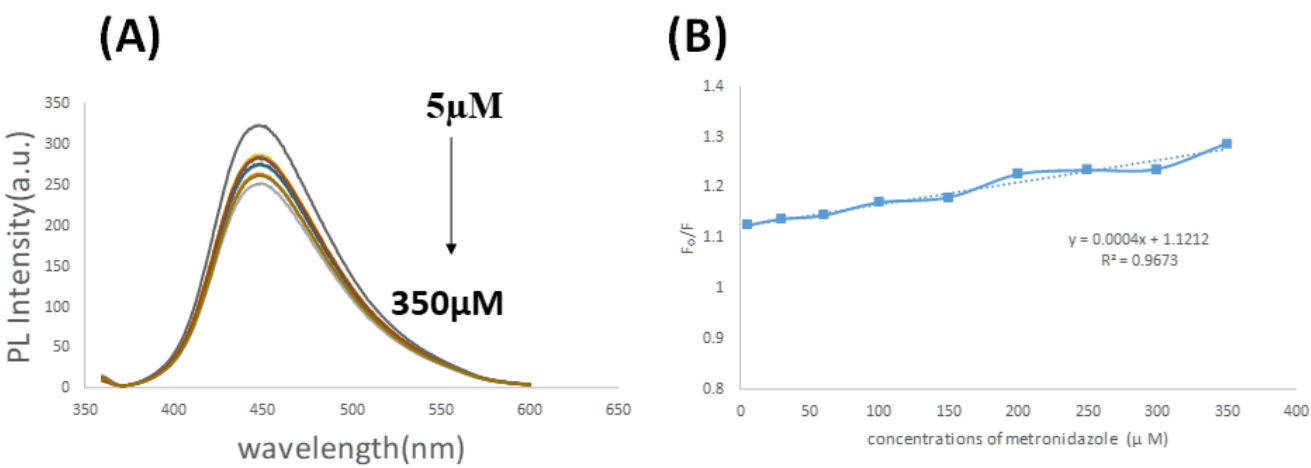


Fig. 5

Figure 5

A) Change in the fluorescence intensity of the R-CQDs compound in the presence of different concentrations of metronidazole from 5.0 to 350.0 μM. B) Increasing the relative sensitivity of the detection system with different concentrations of metronidazole, 5.0 to 350.0 μM

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