

Determination of ciprofloxacin using the Ziziphus mauritania derived fluorescent carbon dots based optical sensor

Navneet Chaudhry

Delhi Technological University

Damini Verma

Jawaharlal Nehru University

Amit K Yadav

Jawaharlal Nehru University

Jai Gopal Sharma

Delhi Technological University

Pratima Solanki (✉ pratimarsolanki@gmail.com)

Jawaharlal Nehru University

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Abstract

The rapid determination and sensitive monitoring of antibiotics residues are critical challenges and highly essential for the protection of human and environment. Hence, in the present work, facile green synthesis of fluorescent carbon quantum dots (CDs) has been done using one step hydrothermal treatment of fruit exact of *Ziziphus mauritiana* for the first time, acting as natural source of carbon for optical determination of ciprofloxacin (CIP). These prepared CDs was used to selectively detect CIP, exhibiting high quantum yield of 30% at excitation wavelength of 340 nm. After the optimal parameters, the sensor gave the linear detection range from 20 to 100 μM along with limit of detection of 9.20 μM . The current work depicts the potential to effectively and rapidly determine other forthcoming environmental pollutants.

1. Introduction

Fluoroquinolones (FQs) comes under the class of antibiotics that are extensively used as human and veterinary medicines for fighting against many Gram positive (+) and gram negative (-) bacteria (Lavaee et al. 2017). Ciprofloxacin (CIP) is the most frequently used antibiotics among FQs that is oftenly utilize for treating animal and human diseases. (Picó and Andreu 2007) They are highly engaged in agricultural, medical and other areas owing to its high-cost efficiency and antimicrobial properties (Aldred et al. 2013) (Ionescu et al. 2007). But antibiotics are not completely digested by the body, a portion of their active component is discharged into the environment *via* wastewater (Borba et al. 2018)(Chaudhary et al. 2021). The leftover CIP might be transferred to the human body through the food chain and accumulate there in the system. In order to protect human health, numerous organisations or governments have established maximum residue limits. If a huge proportion of these residues stay in the body, it can cause a variety of negative responses as well as diseases, including pneumonia, respiratory tract infection, and cancer (Yu et al. 2017)(Zhang et al. 2014). As a result, it is critical that CIP should be detected using a cost effective, easy, environmentally friendly, and sensitive technique.

Many approaches have been investigated for CIP determination such as electrochemical techniques (Hu et al. 2018), colorimetry (Lavaee et al. 2017), capillary electrophoresis (Montes et al. 2014), high-performance liquid chromatography (Jin et al. 2016), and so on. However, these approaches need complex procedures, lengthy, are costly, and extensive sample processing. On contrast, the fluorescence sensor or optical sensor approach offers the benefits of non-destructive, simple, and cost effective (Li et al. 2019). Owing to its rapid, lack of pre-processing, and high sensitivity detection, fluorescence based optical sensors have been extensively applied to detect many analytes that includes biomolecules (Chai et al. 2021); proteins (Xue et al. 2021); and metal ions (Wang et al. 2021).

Carbon dots (CDs) are suitable alternatives for constructing fluorescent probes ascribing to its exceptional features, such as superb optical qualities (Wang et al. 2017)(Ramezani et al. 2018), water solubility, high chemical stability, and great biocompatibility (Gao et al. 2018)(Liu et al. 2019). There are widely employed in various fields such as medication administration, metal ion sensing, biological

imaging (Atchudan et al. 2019), biosensors (Anh et al. 2017), as well as in other domains (Zong et al. 2014). However, preparation of CDs typically entails the employment of chemical agents such as powerful oxidizers or acids, which can pose a risk to human health and pollute the environment. The hydrothermal approach was often employed by researchers since it is an environmentally friendly, facile, one-step procedure with a cheap synthesis cost than other methods (Hua et al. 2017). The hydrothermal technique of carbonization at low temperature with self-generated pressure is a green approach since no pollutant is emitted into the environment. To synthesize fluorescent CDs, several natural products such as tejapata leaves (Chaudhary et al. 2022), orange juice (Sahu et al. 2012), amino acids (Pei et al. 2015) etc. were employed as precursors. Therefore, it becomes critical to look into environmentally friendly, green and new precursors for the cost-effective preparation of fluorescent CDs. The use of green plant or ecologically friendly resources in the hunt for innovative precursors for CDs is becoming increasingly appealing. However, CDs quantum yield obtained from green resources is usually low (Feng and Zhang 2019)(Dager et al. 2019). In the hunt for achieving high quantum yield, we explored the CDs synthesis from natural precursor i.e., fruit extract of *Ziziphus mauritiana* as a source of carbon. *Ziziphus mauritiana* popularly recognized as Indian jujube,^[1] or Indian plum is a fruit tree species that belongs to Rhamnaceae family. For centuries, it has been used as a herbal treatment. Its fruits that are rich in antioxidant property and valuable medical leaves makes this tree more attractive for providing economical products (Rahman et al., 2021). Hence, the goal of this study is to implement and create a reliable technique for detecting CIP utilising *Ziziphus mauritiana* derived fluorescent CDs as a fluorescent agent.

In the present research, a fluorescent CDs derived from *Ziziphus Mauritania*' fruit extract were used for the optical detection of CIP synthesized *via* green methodology using one step hydrothermal treatment. The entire characterization of CDs was done and examined using X-ray diffraction (XRD), UV-Visible (UV-Vis), Fourier transform infrared (FTIR) and photoluminescence (PL) spectroscopy and scanning electron microscope (SEM). The fabricated CIP optical sensor was developed using the technique of PL and preparing CDs from chemical-free green source for the first time. The effect of CIP concentrations on the CDs PL intensity as well as the selectivity of CIP against various interferents were investigated. These CDs exhibits linearity from 20 to 100 μM for CIP determination, give blue emission along with high water solubility.

2. Experiments

2.1 Chemicals and Instrumentation

The fruit of *Ziziphus mauritiana* (Ber) were obtained from the nearby market of New Delhi, CIP, and other antibiotics were procured from SRL. All chemical reagents were utilized as such without pre-treatment. The crystal structure of CDs was done using X-ray Diffractometer (Miniflex, Rigaku). The investigation of the various functional groups is done through Fourier transformation infrared spectroscopy (FTIR), Perkin Elmer. The fluorescence as well as absorbance of CDs were performed employing the fluorescence spectroscopy, (Aligent Technologies, Cary Eclipse) and UV-Visible spectroscopy (T90 + UV/VIS

Spectrometer), respectively. The elemental composition and morphology of CDs were performed using the energy-dispersive X-ray (EDS) spectroscopy and scanning electron microscope (SEM), JEOL, respectively.

2.2 Synthesis of Carbon Dots

The preparation of fluorescent CDs was done using a facile green route through one step hydrothermal treatment. For this, 40 g of *Ziziphus mauritiana* fruit was weighed and cut it into small pieces. The small pieces were then grinded and converted into paste that was diluted by dissolving it in 50 mL of distilled water to make the fruit extract. Next 20 mL of the fruit extract of *Ziziphus mauritiana* was poured in a Teflon-equipped stainless steel autoclave reactor and kept in muffle furnace for hydrothermal reaction to take place for 5 h at 190°C. Later after cooling the autoclave, centrifugation was done for 20 min at 8000 rpm for obtaining the dark coloured supernatant liquid (Kim and Kim 2018). Moreover, the supernatant was dialyzed *via* the dialysis membrane in 1 L of distilled water for 12 h with 3.5 KDa porosity in which distilled water was replaced in every 30 min. At last, yellowish-brown liquid was collected carrying the fluorescent CDs using a micropipette and placed in fridge 4°C for future experiment. Scheme 1 illustrates the preparation of CDs from *Ziziphus mauritiana* by low temperature, one step hydrothermal reaction followed by the detection of CIP *via* fluorescence enhancement of CDs.

3. Results And Discussion

The quantum yield of CDs derived from fruit extract of *Ziziphus mauritiana* was estimated at excitation wavelength of 340 nm in distilled water employing quinine sulfate as a reference. It was found that CDs yielded 30% of quantum yield from hydrothermal reaction which is much higher as expected from the others natural precursors of CDs. The possible reason for high yield could be ascribed to the high pressure as well as temperature conditions formed during the hydrothermal process providing well-organized carbonization of fruit extract.

The crystal structure of CDs was investigated via XRD. Figure 1 (a) depicts the XRD pattern showing a broad peak at 25.4°, which specifies the interlayer spacing of (002) plane characteristic of graphitic diffraction pattern (Chaudhary et al. 2020).

The functional group was performed using FTIR present in CDs [Fig. 1 (b)]. The FTIR of CDs shows characteristic peaks at 1507 and 3289 cm^{-1} that are ascribed to N-H and N-H/O-H stretching, respectively. This depicts the existence of amino functional groups present on the surface of CDs (Liang et al. 2013). Also, other peaks such as 1020, 1697, 1736, and 2932 cm^{-1} are accredited to C-O, C-C, C=O, C-H stretching vibrations, respectively (Mehta et al. 2014). The strong peaks at 1388 and 1595 cm^{-1} signify the symmetric and asymmetric stretching vibrations, respectively corresponding to the carboxylate anions (Mandal et al. 2012).

The dependence of PL intensity of CDs on excitation wavelength is depicted in Fig. 2 (a). The PL emission spectra of CDs at different excitation wavelengths ranging from 320 to 400 nm as shown in

Fig. 2 (b) (Liang et al. 2013). From this figure, an intense emission peak can be seen at 450 nm with 340 nm excitation wavelength. It is ascribed that at 340 nm wavelength, highest number of CDs are excited. Moreover, the surface characteristic of CDs could also be accountable for excitation-dependent fluorescence performance

Figure 2 (c) display the CDs UV-Visible absorption spectra. In this figure, two peaks are visible. One intense absorption peak at 284 nm and other minor shoulder peak at 250 nm that corresponds to $n\text{-}\pi^*$ transitions of the C-O and $\pi\text{-}\pi^*$ transitions of the C-C bond, respectively (Hu et al. 2014)(Zhang et al. 2012). The inset depicts the CDs in the aqueous suspension in daylight and exhibiting bright blue emission under UV light.

The pH response was performed in order to see the change in fluorescence intensity of synthesized CDs as displayed in Fig. 2 (d). For this experiment, various pH ranging from 2 to 12 was taken to observe change in fluorescence. The data depicts that maximum fluorescence was observed at pH 7.0 of CDs derived from fruit extract of *Ziziphus mauritiana*.

The SEM-EDS was performed to examine its morphology and find the composition of elements present in it, respectively [Fig. 3 (a,b)]. The EDS showed nine elements for example carbon (40.7wt.%), calcium (0.7wt.%), oxygen (41.8wt.%), nitrogen (1.2wt.%), potassium (6.9 wt.%), chlorine (2.8 wt.%), sulfur (0.07wt.%), silicon (4.4 wt.%), and sodium (1.0wt.%) as depicted in Fig. 3 (a). The existence of nitrogen (N) peak in the EDS depicts the nitrogen-doped CDs in which N atoms are provided by the amino acids present in the fruit extract of *Ziziphus mauritiana*. Also, the oxygen (O) element could be ascribed to alkoxy, and hydroxy groups present on the CDs surface.

Figure 3 (a) EDS of CDs synthesized from fruit extract of *Ziziphus mauritiana*

The SEM image of CDs shows that they are spherical in shape. Moreover, the confirmation of EDS results was done using elemental mapping study. Figure 3 (b) shows the composition of nine elements in CDs, designated by different colors (C, O, Ca, K, S, Si, Cl, Na, and N).

3.1 Detection of Ciprofloxacin using Carbon Dots

The interaction of different CIP concentrations on CDs, PL intensity data is depicted in Fig. 4 (a). For this experiment, CIP concentrations from 1 to 100 μM were added in solution which lead to enhancement in fluorescence intensity as can be seen from the PL spectra at 448 nm excitation wavelength. This could be ascribed to the production of a complex formed between the amine group of CDs and carboxylate group of CIP. Also, a charge transfer from CDs to CIP can enhance the PL intensity (Kim and Kim 2018). Moreover, the increase in PL intensity of CDs could also be accredited to the hydrogen bonding interactions, as the CDs comprise of hydroxyl and carboxyl groups as confirmed from FTIR analysis that provides aqueous solubility to bind with CIP *via* hydrogen bonding.

Figure 4 (b) represents a relation between the PL intensity of CDs with concentrations of CIP. A good linearity can be seen between CIP concentrations and PL intensity of CDs ranging from 20–100 μM having regression coefficient (R^2) of 0.982 and LOD of 9.20 μM . The LOD was estimated using the standard formula of $3\sigma/S$ (where, σ depicts the linear curve slope and S is the standard error of intercept). This confirms that CIP could be determined from the enhancement in fluorescence of CDs.

3.2 Selectivity analysis of Carbon Dots against Ciprofloxacin

The CDs selectivity was performed utilizing different potential interfering chemicals for CIP determination [Fig. 4 (c)]. For this study, various antibiotics such as gentamycin, ampicillin, amoxicillin, tetracycline, moxifloxacin, levofloxacin and norfloxacin were taken, added one by one and its PL intensity was noted. Among all, only CIP exhibited a very intense fluorescence enhancement that displayed that CDs derived from *Ziziphus mauritiana* fruit extract have high selectivity towards it, enabling the determination of CIP against other interfering antibiotics.

4. Conclusions

In conclusion, a chemical-free synthesis procedure using *Ziziphus mauritiana* fruit extract as a carbon-rich source was used to develop an efficient and simple CDs in the aqueous medium. The characterizations of the green, fluorescent CDs were well confirmed and supported by XRD, FTIR, UV-Vis and PL for fluorescence sensor applications. The intense blue emission of CDs displayed good aqueous medium stability. The green synthesised CDs demonstrated remarkable selectivity for CIP determination among the other analytes examined by enhancement in fluorescence intensity having quantum yield of 30%. As a result, the fluorescent based optical sensor exhibited wide linearity from 20 to 100 μM along with LOD of 9.20 μM . The synthesised CDs possesses the benefits of selectivity and therefore can bind to the CIP *via* the functional group present on CDs surface. The developed synthesis procedure of CDs derived from the fruit extract of *Ziziphus mauritiana* is highly economically feasible, greener, convenient and could be utilized for detecting other antibiotics in future.

Declarations

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Author Contributions

Navneet Chaudhary, Damini Verma: conceptualization, formal analysis, methodology, software, visualization, data curation, writing— original draft, writing—review and editing. **Damini Verma:**

methodology, data curation, writing—review and editing. **Amit K Yadav**: writing— original draft, writing— review and editing; **Navneet Chaudhary**: investigation. **Jai Gopal Sharma**: supervision, writing— review and editing, resources. **Pratima R Solanki**: project administration, conceptualization, methodology, supervision, writing— review and editing, funding acquisition and Editing

Ethical approval: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable.

Competing interests: The authors declare no competing interests.

Data availability: The datasets utilized or/and investigated during the present study are available from the corresponding author on reasonable request.

References

- Aldred KJ, McPherson SA, Turnbough Jr CL, et al (2013) Topoisomerase IV-quinolone interactions are mediated through a water-metal ion bridge: mechanistic basis of quinolone resistance. *Nucleic Acids Res* 41:4628–4639
- Anh NTN, Chowdhury AD, Doong R (2017) Highly sensitive and selective detection of mercury ions using N, S-codoped graphene quantum dots and its paper strip based sensing application in wastewater. *Sensors Actuators B Chem* 252:1169–1178
- Atchudan R, Edison TNJI, Perumal S, et al (2019) Green synthesized multiple fluorescent nitrogen-doped carbon quantum dots as an efficient label-free optical nanoprobe for in vivo live-cell imaging. *J Photochem Photobiol A Chem* 372:99–107
- Borba FH, Schmitz A, Pellenz L, et al (2018) Genotoxicity and by-products assessment in degradation and mineralization of Ciprofloxacin by UV/H₂O₂ process. *J Environ Chem Eng* 6:6979–6988
- Chai S, Zan G, Dong K, et al (2021) Approaching superfoldable thickness-limit carbon nanofiber membranes transformed from water-soluble PVA. *Nano Lett* 21:8831–8838
- Chaudhary N, Gupta PK, Eremin S, Solanki PR (2020) One-step green approach to synthesize highly fluorescent carbon quantum dots from banana juice for selective detection of copper ions. *J Environ Chem Eng* 8:103720
- Chaudhary N, Verma D, Sharma JG, Solanki PR (2022) A novel bioinspired carbon quantum dots based optical sensor for ciprofloxacin detection. *Mater Lett* 308:131090

- Chaudhary N, Yadav AK, Sharma JG, Solanki PR (2021) Designing and characterization of a highly sensitive and selective biosensing platform for ciprofloxacin detection utilizing lanthanum oxide nanoparticles. *J Environ Chem Eng* 9:106771
- Dager A, Uchida T, Maekawa T, Tachibana M (2019) Synthesis and characterization of mono-disperse carbon quantum dots from fennel seeds: photoluminescence analysis using machine learning. *Sci Rep* 9:1–12
- Feng X, Zhang Y (2019) A simple and green synthesis of carbon quantum dots from coke for white light-emitting devices. *Rsc Adv* 9:33789–33793
- Gao W, Song H, Wang X, et al (2018) Carbon dots with red emission for sensing of Pt^{2+} , Au^{3+} , and Pd^{2+} and their bioapplications in vitro and in vivo. *ACS Appl Mater Interfaces* 10:1147–1154
- Hu C, Yu C, Li M, et al (2014) Chemically tailoring coal to fluorescent carbon dots with tuned size and their capacity for Cu (II) detection. *Small* 10:4926–4933
- Hu X, Goud KY, Kumar VS, et al (2018) Disposable electrochemical aptasensor based on carbon nanotubes- V_2O_5 -chitosan nanocomposite for detection of ciprofloxacin. *Sensors Actuators B Chem* 268:278–286
- Hua J, Yang J, Zhu Y, et al (2017) Highly fluorescent carbon quantum dots as nanoprobes for sensitive and selective determination of mercury (II) in surface waters. *Spectrochim Acta Part A Mol Biomol Spectrosc* 187:149–155
- Ionescu RE, Jaffrezic-Renault N, Bouffier L, et al (2007) Impedimetric immunosensor for the specific label free detection of ciprofloxacin antibiotic. *Biosens Bioelectron* 23:549–555
- Jin T, Wu H, Gao N, et al (2016) Extraction of quinolones from milk samples using bentonite/magnetite nanoparticles before determination by high-performance liquid chromatography with fluorimetric detection. *J Sep Sci* 39:545–551
- Kim K, Kim J (2018) Synthesis of carbon quantum dots from jujubes for detection of iron (III) ions. *J Nanosci Nanotechnol* 18:1320–1322
- Lavaee P, Danesh NM, Ramezani M, et al (2017) Colorimetric aptamer based assay for the determination of fluoroquinolones by triggering the reduction-catalyzing activity of gold nanoparticles. *Microchim Acta* 184:2039–2045
- Li Z, Cui Z, Tang Y, et al (2019) Fluorometric determination of ciprofloxacin using molecularly imprinted polymer and polystyrene microparticles doped with europium (III)(DBM) 3phen. *Microchim Acta* 186:1–10

- Liang Q, Ma W, Shi Y, et al (2013) Easy synthesis of highly fluorescent carbon quantum dots from gelatin and their luminescent properties and applications. *Carbon* N Y 60:421–428
- Liu ML, Chen B Bin, Li CM, Huang CZ (2019) Carbon dots: synthesis, formation mechanism, fluorescence origin and sensing applications. *Green Chem* 21:449–471
- Mandal A, Dandapat A, De G (2012) Magic sized ZnS quantum dots as a highly sensitive and selective fluorescence sensor probe for Ag⁺ ions. *Analyst* 137:765–772
- Mehta VN, Jha S, Kailasa SK (2014) One-pot green synthesis of carbon dots by using *Saccharum officinarum* juice for fluorescent imaging of bacteria (*Escherichia coli*) and yeast (*Saccharomyces cerevisiae*) cells. *Mater Sci Eng C* 38:20–27
- Montes RHO, Marra MC, Rodrigues MM, et al (2014) Fast determination of ciprofloxacin by batch injection analysis with amperometric detection and capillary electrophoresis with capacitively coupled contactless conductivity detection. *Electroanalysis* 26:432–438
- Pei S, Zhang J, Gao M, et al (2015) A facile hydrothermal approach towards photoluminescent carbon dots from amino acids. *J Colloid Interface Sci* 439:129–133
- Picó Y, Andreu V (2007) Fluoroquinolones in soil—risks and challenges. *Anal Bioanal Chem* 387:1287–1299
- Ramezani Z, Qorbanpour M, Rahbar N (2018) Green synthesis of carbon quantum dots using quince fruit (*Cydonia oblonga*) powder as carbon precursor: application in cell imaging and As³⁺ determination. *Colloids Surfaces A Physicochem Eng Asp* 549:58–66
- Sahu S, Behera B, Maiti TK, Mohapatra S (2012) Simple one-step synthesis of highly luminescent carbon dots from orange juice: application as excellent bio-imaging agents. *Chem Commun* 48:8835–8837
- Wang S, Chen H, Xie H, et al (2021) A novel thioctic acid-carbon dots fluorescence sensor for the detection of Hg²⁺ and thiophanate methyl via S-Hg affinity. *Food Chem* 346:128923
- Wang S, Wang Y, Yang K, et al (2017) Synthesis of carbon dots originated from hydroxypropylmethyl cellulose for sensing ciprofloxacin. *Anal Sci* 33:1129–1134
- Xue J, Yi J, Zhou H (2021) Label-Free Fluorescence Molecular Beacon Probes Based on G-Triplex DNA and Thioflavin T for Protein Detection. *Molecules* 26:2962
- Yu W, Zhan S, Shen Z, et al (2017) Efficient removal mechanism for antibiotic resistance genes from aquatic environments by graphene oxide nanosheet. *Chem Eng J* 313:836–846
- Zhang L, Xing Y, He N, et al (2012) Preparation of graphene quantum dots for bioimaging application. *J Nanosci Nanotechnol* 12:2924–2928

Zhang R, Wang Y, Yu L-P (2014) Specific and ultrasensitive ciprofloxacin detection by responsive photonic crystal sensor. *J Hazard Mater* 280:46–54

Zong J, Yang X, Trinchì A, et al (2014) Carbon dots as fluorescent probes for “off–on” detection of Cu^{2+} and l-cysteine in aqueous solution. *Biosens Bioelectron* 51:330–335

Figures

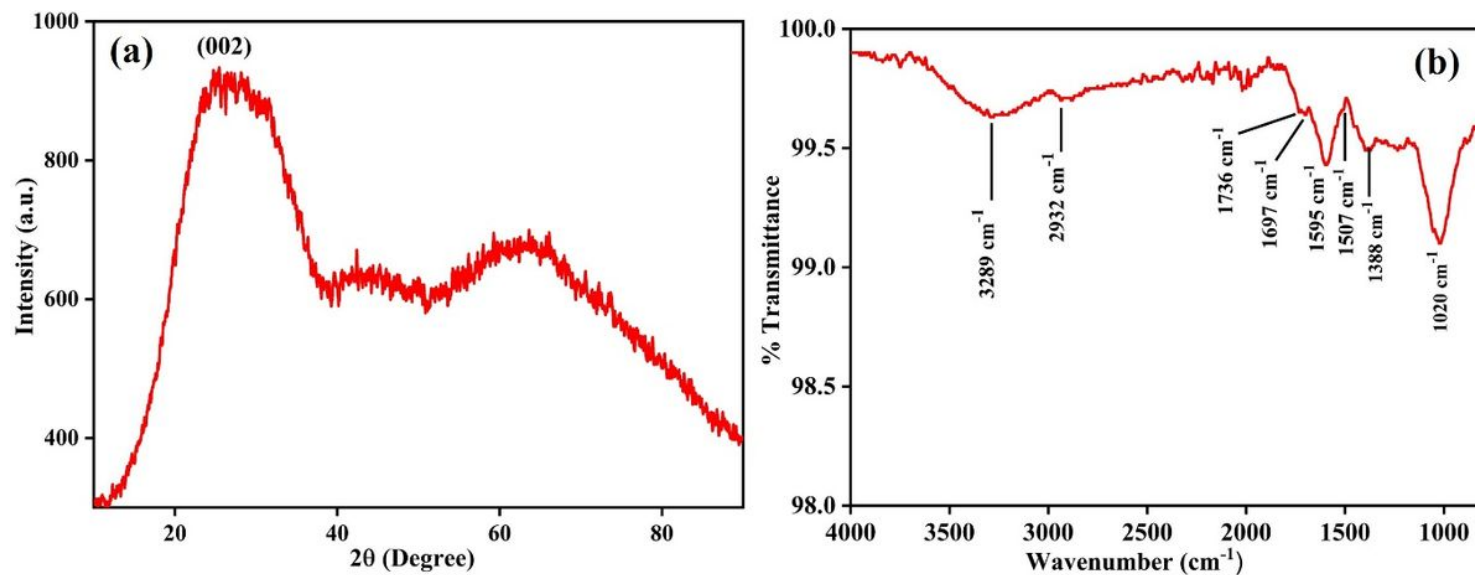


Figure 1

(a) XRD of fluorescent CDs, (b) FT-IR of CDs displaying the various functional groups

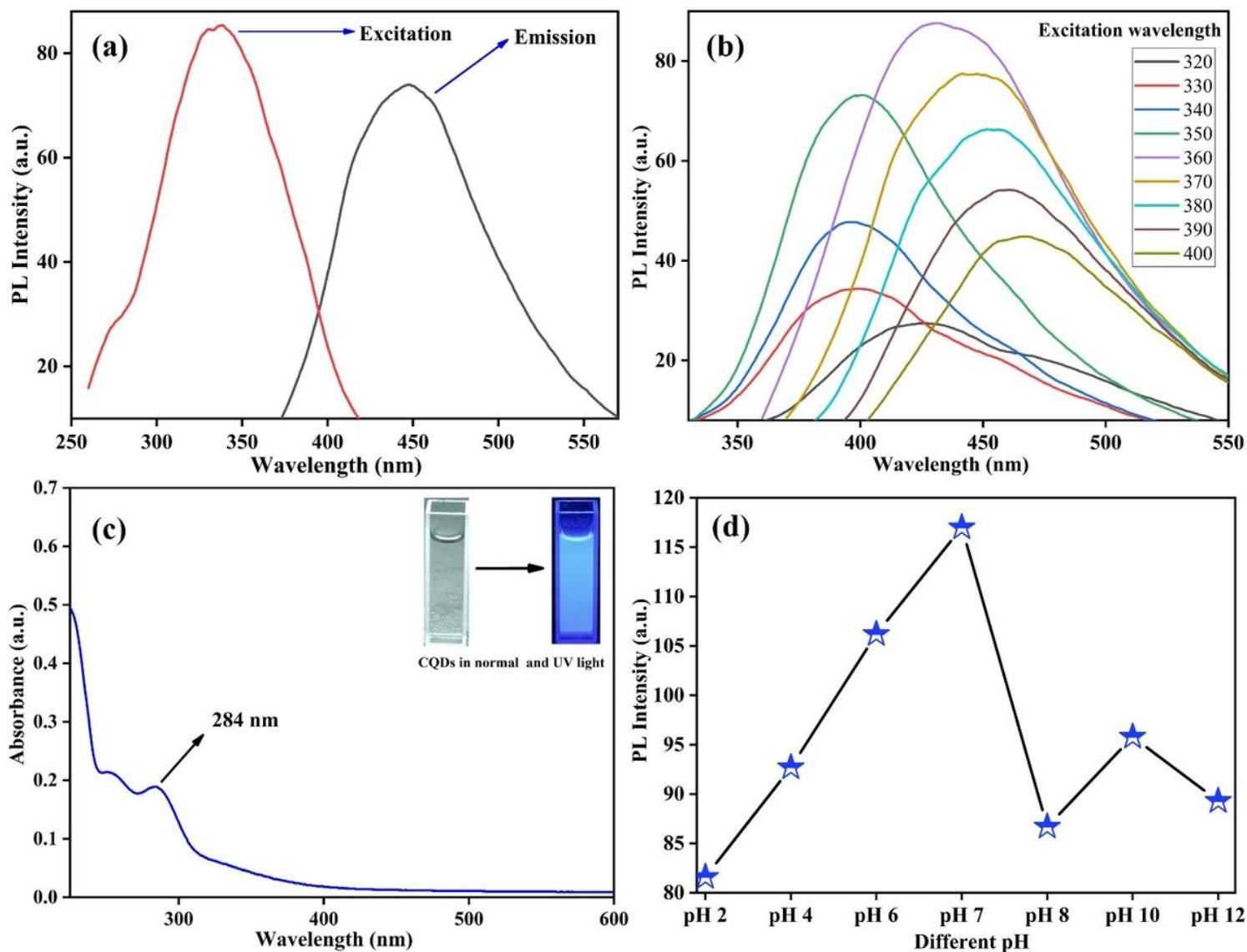


Figure 2

(a) PL spectra showing emission and excitation wavelength of CDs, (b) PL spectra of CDs derived from fruit extract of *Ziziphus mauritiana* with 320-400 nm excitation wavelengths, (c) UV-Visible spectra of CDs, inset shows the fluorescent CDs in daylight and UV light; (d) pH response with change in fluorescence of synthesized CDs

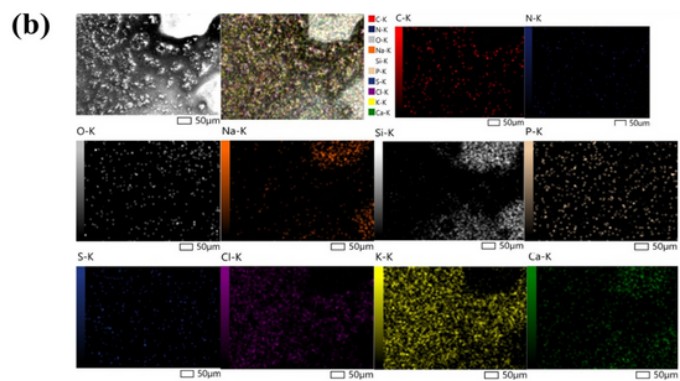
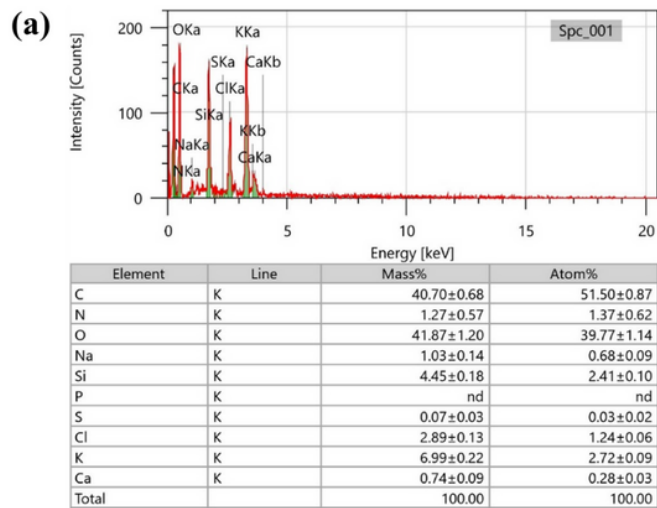


Figure 3

(a) EDS of CDs synthesized from fruit extract of *Ziziphus mauritiana*

(b) SEM image and EDS mapping of CDs

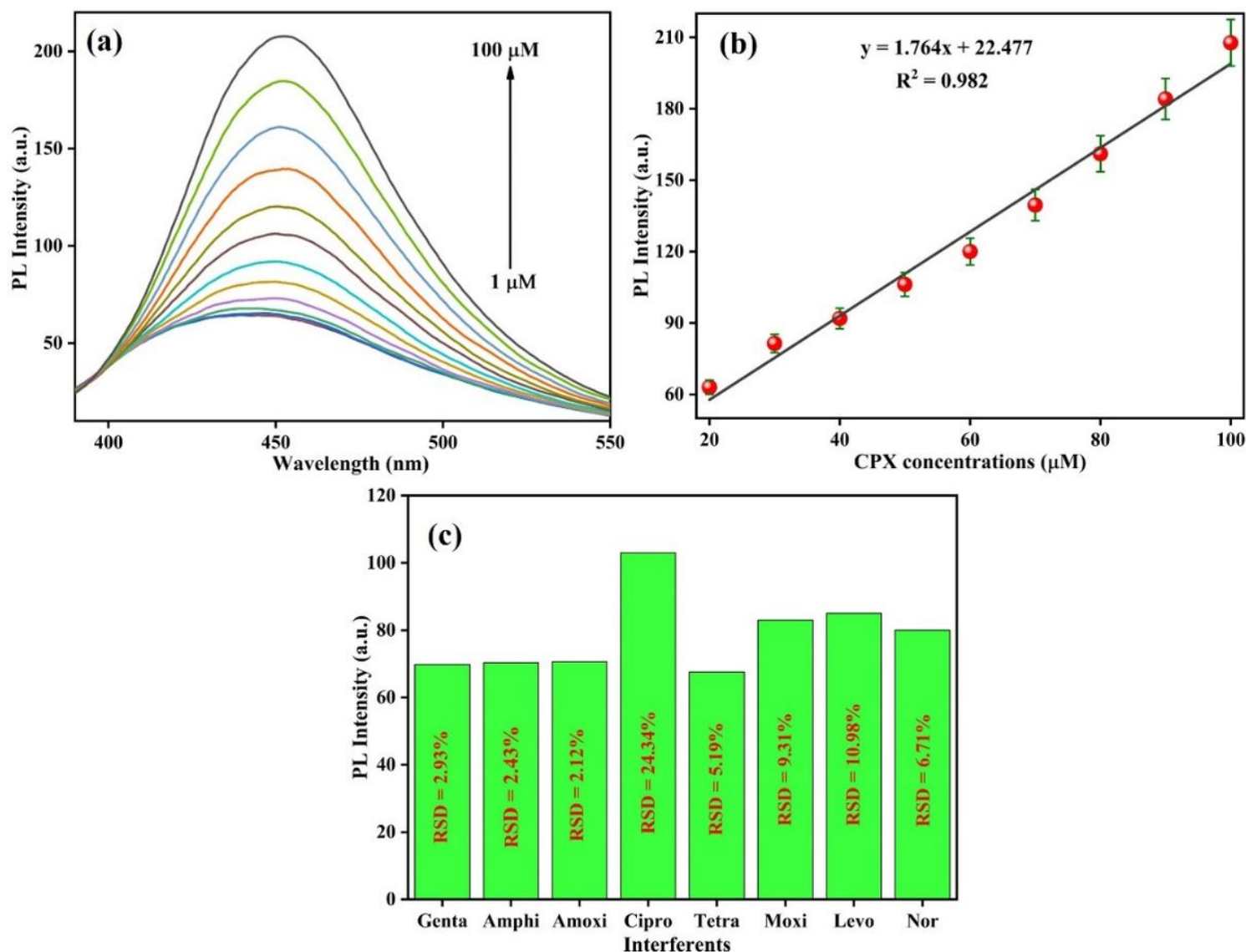


Figure 4

(a) The sensing response between the concentrations of CIP and PL intensity of CDs, (b) Calibration curve between concentrations of CIP and CDs PL intensity and; (c) Selectivity analysis of CDs against other antibiotics

Supplementary Files

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- [Scheme1.jpeg](#)