Europium doped carbon dots for selective and sensitive detection of tetracycline

by

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2023

Thesis submitted to the School of Mining and Geosciences of Nazarbayev University in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Chemistry

Nazarbayev University
2023

Acknowledgements

I am grateful for the support of Professor Timur Atabaev, the laboratory Advanced Nanochemistry, and my colleagues.

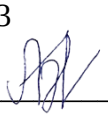
Originality Statement

I, Aigerim Babenova, hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at Nazarbayev University or any other educational institution, except where due acknowledgement is made in the thesis.

Any contribution made to the research by others, with whom I have worked at NU or elsewhere is explicitly acknowledged in the thesis.

I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

Signed on 10.04.2023



ABSTRACT

Tetracycline (TC) overuse in livestock farms has resulted in its widespread presence in water bodies, which can have a negative impact on the ecosystem and human health. Therefore, the aims of current study are to develop rapid, low-cost, shelf-life stable, sensitive, and selective detection method for TC antibiotic in water. As a result, an optical detection agent based on europium-doped carbon dots (Eu-CDs) was synthesized and extensivity characterized by physical-chemical methods. We found that after optimization process, the linear range of TC detection was found to be 0.01-5 μM, with an impressive limit of detection of 6.5 nM. The selectivity analysis with other antibiotics revealed that Eu-CDs are selective to TC only and show negligible response to other TC derivatives. It was shown that optimal pH for TC detection was ~ 7, and Eu-CDs retain high detection ability even in high sodium chloride (NaCl) solution. These results suggest that Eu-CDs can be used as a quick and effective colorimetric agent for TC detection in water samples.

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1. Introduction

1.1 Water pollution

Today, there is an increasing shortage of drinking water, as is evident in countries across Central Asia [1] where water sources have been polluted due to anthropogenic influences. Industrial pollution of water sources with heavy metals, agricultural activities surrounding water bodies, mineralization of rivers, and soil salinization are some of the key factors contributing to this issue. The result is a deterioration of the ecological environment and a negative impact on human health.

Industrial influence leads to heavy metal pollution, making water sources toxic to use without additional filtration. The Shagan River in the Abay region of Kazakhstan is polluted with heavy metals such as iron (Fe), strontium (Sr), manganese (Mn), lithium (Li), and uranium (U) [2]. The concentration of Fe in most samples was around 450 μg/L, with the highest concentration being 563 μg/L [2]. The maximum acceptable concentration for Fe is 300 μg/L [2]. The highest concentration of Sr was 9663 μg/L, while the acceptable concentration is 7000 μg/L [2]. The results for Mn, Li, and U were similar, with concentrations exceeding acceptable levels.

Agricultural activities, such as irrigation and livestock farming, are another cause of water source pollution. The overuse of water for irrigation and livestock farming can lead to mineralization and increased salinity in water sources. For instance, in Uzbekistan, rivers and lakes are affected by mineralization, which results in higher concentrations of nitrogen, phosphorus, and arsenic ions that originate from fertilized agricultural soils [1]. Additionally, the use of antibiotics in livestock farming contributes to water pollution by contaminating the surrounding soil and water resources through animal waste and excretion. Livestock farms often use antibiotics to treat animals and promote muscle growth, which can have harmful environmental effects [3]. Antibiotic residues with heavy metals in the environment can lead to an increase in antibiotic resistance genes (ARG), which can disrupt ecosystem processes such as nitrogen cycling and conservation [3]. For example, the metal complex with tetracycline (TC) is attracted to gram-negative bacteria and accumulates in the periplasmic space [4]. Then, the TC detaches from the metal complex and passes inside the cell, creating ARG. Gram-positive bacteria allow the metal complex with TC to pass into the cytoplasm due to diffusion and the different concentrations of metals inside and outside the cage and the pH

[4]. Therefore, it is necessary to monitor water pollutants to avoid negative impacts on the environment and human health.

1.2 Tetracycline. Properties and toxicological affect

TC is a broad-spectrum antibiotic used to treat bacterial infections in both humans and animals. TC exhibits antibacterial properties against a variety of infections. It is effective against respiratory tract infections, urinary tract infections, and skin bacterial diseases [5]. Additionally, it can also treat sexually transmitted infections. It is also commonly used as a growth stimulant supplement in livestock farms [6,7]. TC has the ability to affect both gram-positive and gram-negative bacteria due to its unique chemical structure. The TC molecule contains an electron-deficient part, consisting of the amino and enone groups, as well as an electron-rich part, containing the phenolic ring [6]. Because of its structure, TC is able to bind to metal ions to form complexes under pH influence. At low acidic pH, TC molecules become cations [6]. At neutral pH, TC exhibits a zwitterion as it loses protons on the phenol ketone group [6]. At high pH levels above 7.7, TC becomes an anion [6].

Although TC has beneficial properties, it is not fully absorbed by the body, and between 69% and 86% of the antibiotic is excreted through urine and feces [7]. As a result, TC contamination can originate from various sources, including the excreta of humans and animals treated with the drug, its use as an additive in livestock feed and the resulting excrement, medical waste such as expired antibiotics and tools contaminated with the drug, as well as waste from pharmaceutical industries [6,7,8,9]. This contamination can have detrimental effects on the environment, leading to toxicity for both humans and the ecological system.

For example, antibiotics from the TC group that are found in the soil are perfectly absorbed by plants. As a result, chlortetracycline (CTC), TC, and oxytetracycline (OTC) are absorbed by plant roots and transported to stems and leaves [6]. A study was conducted to investigate the effect of TC on the growth of wheat. As the concentration of TC in wheat roots increased, root growth and wheat growth were inhibited [10]. In the presence of TC, wheat growth was reduced by 7-73% compared to control wheat without TC [10]. Additionally, the amount of chlorophyll in wheat decreased by 20-98% with an increase in the concentration of TC, which had the same effect on the number of carotenoids in wheat [10]. In addition to the previously mentioned effect on chlorophyll and carotenoid levels in wheat, research on rice growth has shown that the bark biomass is reduced under the influence of tetracycline at concentrations ranging from 5 to 30 mg/L [6]. Furthermore, studies on green algae have

demonstrated that tetracycline negatively affects the chloroplast system, plasmolysis function, and starch formation [6]. Oxidative stress was also observed in cyanobacteria and protozoan green algae [6].

In larger living organisms, such as flies and worms, the mitochondria are also impacted, disrupting the production of oxygen in cells and potentially leading to growth failure [11] and reduced fertility [11]. TC toxicity has been observed to cause various adverse effects, such as embryotoxicity. For instance, in carp, concentrations of TC ranging from 90 to 900 µg/L resulted in developmental abnormalities, such as the impairment of the tail and spine formation, scoliosis, and structural defects in the chord, cardiac system, and edema [6]. Similarly, zebrafish embryos [6,12] exposed to a concentration of 20 µg/L of TC showed developmental delay and oxidative stress. Additionally, the growth and hatching process of mature larvae were inhibited, and mutational cell destruction was prevented. TC has also been linked to genotoxicity and disruption of earthworm growth, leading to DNA changes [6]. In rats, a concentration of 200 µg/kg of tetracycline caused toxicity, resulting in liver and pancreatic dysfunction at lower concentrations of 50 µg/kg [6].

A study of the water source of the Wan River in Anhui Province, China showed that TC in water is of medium or high risk [13]. Even low concentrations of TC can lead to the development of antibiotic resistance genes (ARGs). An example of such genes is TC-resistant genes that form in pig farms due to additives in feed. As a result, this negatively affects the ecosystem and human health [14]. Similarly, the use of antibiotics in fish breeding ponds can also lead to the development of ARGs. For example, TC-resistance genes TC(A) and TC(C) were discovered on fish farms in Zhongshan, China [15]. The concern is that even the simplest bacteria can transfer ARGs to each other, posing a serious threat to the ecosystem. Another study found that the wastewater treatment plant contains eight TC resistance genes, namely TC(A), TC(B), TC(C), TC(E), TC(M), TC(O), TC(S), and TC(X) [16]. In addition, the concentration of TC in the untreated wastewater entering the plant was measured to be 336 ng/L [16]. Therefore, it is not recommended to discharge this wastewater into reservoirs, rivers, or other water sources.

Furthermore, in addition to the aforementioned points, there is the phenomenon of biomagnification, which involves the transfer of TC from animal products such as meat, milk, fish, and water into the human body. For example, the study showed that fish tissue contained oxytetracycline was higher than the 0.1 ng/g allowed concentration [17]. There were 3 types of fish which were cultured tilapia, wild catfish, and cultured catfish. The result showed that wild

catfish had 0.106 μg/g [17], while cultured tilapia and catfish had a higher concentration of OTC which were 0.147 and 0.313 μg/g [17]. And the only grill method of cooking can reduce the concentration of OTC in fish [17]. A study was conducted using a simulator of the intestinal environment to examine the effect of chronic exposure to TC [18]. The study found that a dose of 150 ng/mL reduced the number of anaerobic bacteria within 7 days [18]. However, the study did not investigate the consequences of this decrease. Another study examined the gut microbiota in the feces of three patients [19]. It found that when the TC concentration reached 0.15 μg/mL, the proportion of Bacteroides bacteria increased from 1.68% to 5.70% and 5.13% to 13.50% in two patients' samples after 40 days [19]. In the third patient, Clostridium bacteria increased from 3.50% to 25.34%, which can cause diarrhea [19]. TC resistance genes, including TC(O), TC(Q), TC(W), and TC(X), were detected in all three patients, likely due to their long-term treatment with TC antibiotics [19]. Another study investigated the effect of TC on the integrity and function of human intestinal epithelial cells as a barrier [20]. The results showed that barrier disruption occurred at 15 and 150 μg/mL after 24 and 48 hours, respectively [20]. This was evidenced by the translocation of labeled bacteria from the apical to the basal location. In addition to the risk of tetracycline entering the human body, the risk increases with the consumption of drinking water in areas located near livestock farms [25]. This is because the soil fertilized by manure contains tetracycline, which is then transported to nearby water sources [25].

Thus, TC is a widely used antibiotic for medical treatment in humans and animals, as well as in dietary supplements due to its growth-promoting effects. However, its use can also lead to environmental pollution through animal and human experiments, as the drug is poorly absorbed by the body. The consequences of this pollution include disruption of the growth and development of algae, small insects, small fish, and their embryos, the spread of tetracycline-resistant genes in drinking water and wastewater, impacts on human intestinal microbiota such as an increase in harmful bacteria or a decrease in beneficial anaerobic bacteria, and damage to intestinal epithelial cells.

1.3 Conventional methods of TC detection

Due to the use of TC in livestock farms and its release with excrement, the environment surrounding these farms becomes polluted. High-performance liquid chromatography (HPLC) and mass spectrometry (MS) is the most commonly commercial method for studying and determining TC in water. For example, research group studied 3 rivers of Hong-Kong, which are Yuen Long, Kam Tin, and Shing Min [21]. The researchers measured the concentrations of antibiotics in water using MS system combined with HPLC. The results demonstrate that the upper and middle parts of Yuen Long River have high concentrations of TC (222.2 ng/L) [21]. The same trend is observed in the upper and middle parts of Kam Tin River, but with a higher concentration of chlortetracycline (CTC) (220.2 ng/L) compared to TC [21]. Additionally, Kam Tin River has a higher concentration of oxytetracycline (OTC) in its middle part [21]. In contrast, results showed high concentrations of TC (112 ng/L) in Shing Mun River and no presence of CTC and OTC [21]. OTC and CTC are antibiotics that belong to the tetracycline group. The researchers mentioned pig manure and activity of stock raising as the source of TC contamination. An additional example is the tributary of the Jiyun River in Beijing, China. Researchers Li et al. studied the water surface, sediment, and suspended particulate matter of the Jiyun River [22]. They found the presence of the antibiotic OTC in all three samples from the river. However, the highest concentration of OTC was observed in a tributary near a livestock activity area [22]. The results showed that the average concentration of TC in May was 2.1 ng/L and that of OTC was 16.12 ng/L. In December, the average concentrations were 1.09 ng/L (TC) and 10.78 ng/L (OTC) [22]. TC has a lower concentration compared to OTC in water due to its high adsorption to sediment. Contamination of the tributary of the Jiyun River leads to contamination of the main Jiyun River due to the dispersal of antibiotics within it.

One more study about TC contamination of the Yangtze River which is one of the primary sources of freshwater in China. The lower part of the river supplies fresh water to the developed and densely populated provinces of Jiangsu and the municipality of Shanghai, where about 88 million people live [23]. The dense population has led to the consequence of anthropogenic water pollution due to waste of municipal sewage, and industrial and agricultural wastewater. Therefore, the quality of the drinking water from the river is become concerning. Because the general antibiotic concentrations in agricultural soils near the Yangtze River increased from 4.55 to 2010 ng/L [23]. Therefore, researchers Wang et. al. investigated the quantity of presence of TC antibiotics and their derivatives OTC and doxycycline (DOX) in

the river with fresh water and determined which of the sources of wastewater had a greater impact on contamination [23]. Additionally, they observe the quantity of the antibiotics during dry (January), normal (July) and flood (October) seasons. The research team analyzed antibiotics (TC, CTC, and DOX) and using the ultra-performance liquid analytical method chromatography coupled with paired-ion electrospray ionization-triple quadrupole tandem mass spectrometry [23]. According to the result, there was an observed trend in the seasons such as the highest concentration of antibiotics was in the dry season than in the normal season and the lowest concentrations were in the wet season. Therefore, TC concentration was 11.16 ng/L, 18.98 ng/L (CTC), and 56.09 ng/L (DOX) [23]. Moreover, some samples of drinking water showed high TC concentrations in some parts of the river such as 30.58 ng/L, 109.87 ng/L, and 68.66 ng/L [22]. According to the contribution of TC contamination observed that the tributaries (74.5%) into rivers have more loading of TC pollution than wastewater (25.5%) [23].

Another research group conducted a study on the land area surrounding the Yuqiao water reservoir, which included animal farms [24]. The researchers observed the migration of antibiotics from animal waste and the density of their distribution. As a result, the authors collected samples of manure, wastewater, soil (both fertilized and unfertilized), feed, and water from pig, cattle, and layer farms [24]. Then for analysis of antibiotics in samples were used ultra-performance liquid chromatography and micro mass triple quadrupole mass spectrometer. The result showed that the detection rate of TC was higher in all types of samples among other antibiotics which is an average of 33.3% in comparison with sulfonamide antibiotics is an average of 3.7% [24]. The reason for the high concentration of TC is a cheap and effective medical treatment for diseases. The concentration DXC derivative of TC is 4201.13 μg/L in pig wastewater, while OTC concentration is 712.16 mg/kg in pig manure [24]. Around pig farms result showed that soil samples had 1845.22 μg/kg of CTC [24]. Also, the authors noted that the high concentration of TC antibiotic group connected with influence on the growth of pigs. According to the result of the distribution density of antibiotics, there is a more contaminated southern area in comparison with the northern area [24]. Due to the flat terrain, larger population, and easier transportation, the south has a more favorable environment compared to the mountainous north, which has more difficult transportation. Furthermore, antibiotics migrate from feed to manure, then to soil, and finally to water [24]. However, the concentration of antibiotics in water decreases after passing through manure and soil due to the influence of bio- and photodegradation [24].

Another research group [25] studied migration TC and their derivatives from manure-fertilized soil to surface water and groundwater near the vegetable farmland in Guangzhou, China. Manure has a high TC content due to low adsorption by animal organisms, and thus, 50-60% of TC is released through urine and feces that are present in manure [25]. The manure used to fertilize the soil was from pig farms that contained from result such concentrations of TC -16.9 mg/kg, OTC - 107.9 mg/kg, and CTC - 54.2 mg/kg [25]. Initial concentration measurements were made to investigate how much antibiotics degrade before entering the aquatic environment. Further manure was mixed with fertilizer and cultivated the land on the vegetable farm. The resulting analysis showed that the cultivated soil had 37.18 mg/m^2 of TC, 237.38 mg/m^2 of OTC, and 119.24 mg/m^2 of CTC [25]. The research group created artificial rain since weather conditions were one of the parameters of influence the migration of antibiotics from soil to water. According to the results taken from the runoff, the concentration of antibiotics after the first rain on day 1 of the study showed the presence of TC - 2.79 μg/L, OTC - 35.97 μg/L, and CTC - 14.48 μg/L [25]. With subsequent rains, the concentration of antibiotics decreased and also depends on the amount of precipitation. For example, more precipitation fell on day 7 than on day 20, and thus the concentration of antibiotics on day 20 (TC -0.12, OTC - 0.71, and CTC - 0.38 μg/L) was higher than on day 7 (TC - 0.06, OTC - 0.49, and CTC - 0.22 μg/L) [8]. The following results are measured in the filtrate at a depth of 30 and 60 cm in the soil after rainfall. The result showed that 1 day after the rainfall concentration of antibiotics in the filtrate in the soil at 30 cm depth was 1.71μg/L of TC, 18.44 μg/L of OTC, and 8.44 μg/L of CTC, and at 60 cm depth in the soil concentration decreased TC 0.40 μg/L, OTC 1.99 μg/L, and CTC 2.66 μg/L [25]. The decrease in the concentration of TC with increasing soil depth is associated with the effective adsorption of TC by soil particles. Additionally, the high soil-water distribution coefficient of TC 420–1030 L/kg also contributes to this phenomenon [25]. Thus, the concentration of tetracycline in soil decreases with an increase in the amount and frequency of rain. However, this compound can still be transported into nearby water sources, posing a potential risk to aquatic organisms and human health.

1.4 Literature review of novel methods of TC detection

Although HPLC combined with a MS is effective for TC determination, one significant drawback is the expensive equipment. Therefore, recent studies aim to reduce the cost of determining TC. One such method is the fluorescent method for determining TC using detecting agents.

The tetracycline detection agent can function as an organic or non-organic compound, and when combined with other compounds, can form a detector with optical properties. For example, metal organic framework (MOF), carbon dots (CDs), nanocomposites, nanoclusters, nanochannels.

This research work focuses on a zinc (Zn) metal-organic framework (MOF) that exhibits a color change in its emission when exposed to TC, attributed to the occurrence of MOF structure aggregation [26]. According to the section on the synthesis method of fluorescent Zn-MOF, the detection agent was prepared using a solvothermal method [26]. First, the powder fluorescent MOF was prepared from the salt of Zn ($Zn(CH_3COO)_2 \bullet 2H_2O$), sodium hydroxide (NaOH), and terephthalic acid (TPA) by dissolving everything in methanol and stirring the mixture. Then, the mixture was poured into the reactor and heated for 20 hours at 140°C. After heating, the solution was washed with methanol several times and then dried in an oven under 60°C.

To use the powder for detecting TC, 40 mg of the powder was mixed with 10 mL of ultra-pure water and stirred to dissolve it, then mixed with each different concentration of TC [26]. Measurements were taken 30 seconds after the reaction of the agent and TC. The results show that under an excitation wavelength of 350 nm, the emission of fluorescent Zn-MOF enhanced in the presence of TC (Figure 1.). According to investigation, the detection agent is selective and have emission spectra peak only in the presence of TC [26]. Additionally, the characterization of fluorescent Zn-MOF indicates that the maximum intensity occurs in a pH range of 5 to 7 [26]. The linear range of TC detection and the limit of detection (LOD) are shown in Table 1. It is important to mention that the linear range of detection is calculated by using the ratiometric mode, which divides the intensity peak of the detection TC wavelength at 520 nm by the peak emission of the fluorescent Zn-MOF at 440 nm.

Table 1. Resent research work of detection TC

Detection agent	Linear range of detection TC	LOD	Reference
FMOF-5	1 - 70 µM	5 nM	[26]
NiNCs – Eu^{3+}	0.1 – 50 µM	25 nM (for TC) 21 nM (for OTC)	[27]
AuNCs@DNA_{C12}	10 nM – 5 µM	4 nM	[28]
Eu^{3+}/ NH_2-MIL-53(Al)	0.5 – 60 µM	0.16 µM	[29]
CDs – Eu^{3+}	0 – 3.5 µg/mL	(5.2 ng/mL) 11.7 nM	[30]
Eu-CQDs	0.5 – 200 µM	0.3 µM	[31]
CDs-AuNCs	0.5 – 40 µM	0.056 µM	[32]
Cu-CDs	2 -32 µM (for TC) 2 – 44 µM (for OTC)	0.17 µM (for TC) 0.16 µM (for OTC)	[33]
R-CDs	3 – 40 µM	38.5 nM	[34]
CDs	0.1–50 µM	0.04 µM	[35]

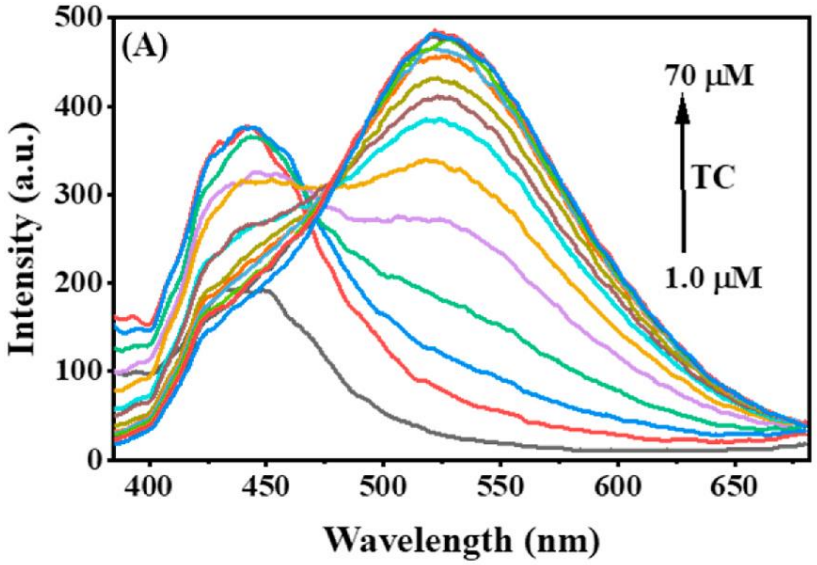

Figure 1. Graph of fluorescent emission spectra peaks of FMOF-5 with addition of different concentration of TC. Source: Adapted with permission from ref. 26. Copyright 2022 Elsevier B.V.

The detectable agent's intensity increases as the tetracycline concentration increases [26]. The mechanism of this enhancement is explained by the adsorption of TC by the agent, as shown in the results of the UV absorption spectrometer [26]. Another result confirming the aggregation of FMOF-5 and TC is the decrease in zeta-potential from 22.2 mV to 19.13 mV

due to electrostatic interaction [26]. Further evidence of aggregation of FMOF-5 with TC is the study of the dried powder of FMOF-5 with TC and FMOF-5 under UV light. The results show that FMOF-5 emits blue light, while FMOF-5 with TC emits yellow-green fluorescent light [26].

Due to the optical properties of the fluorescent agent, it is possible to detect tetracycline using a smartphone under UV lamp with excitation light at 365 nm, as a change in light from blue to green is observed (Figure 2). However, the detection range decreases from 0 to 30 μM, while the linear range of TC detection with measurement on the fluorescent spectrometer is from 0 to 70 μM [26]. The resulting decrease in the limit of detection (LOD) of TC concentration is from 5 nM to 10 nM [26].

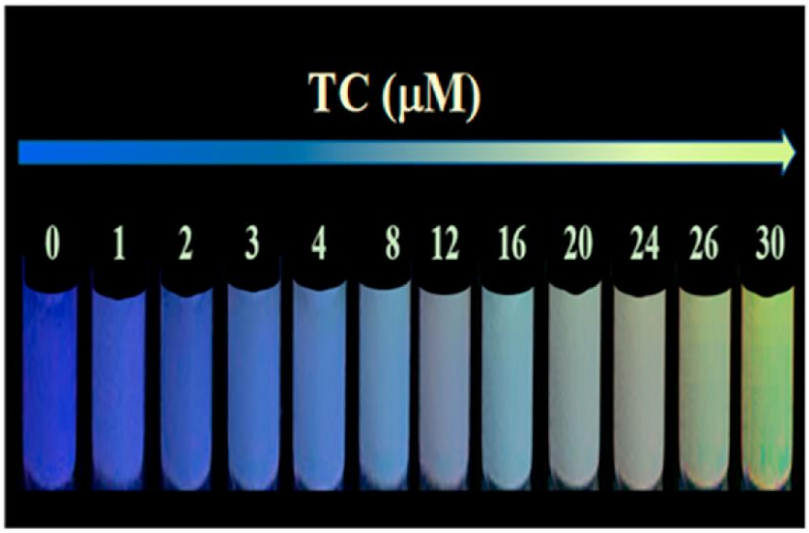

Figure 2. Picture on smartphone of FMOF-5 with addition of different concentration of TC under UV-lamp. Source: Adapted with permission from ref. 26. Copyright 2022 Elsevier B.V.

The detection agent based on nickel nanoclusters (NiNCs) mixed with europium salt (Eu^{3+}) [27]. To prepare NiNCs, polyvinylpyrrolidone (PVP) is dissolved in ultra-pure water with a pH of 10, which is achieved by adding NaOH. Next, L-ascorbic acid (AA) and nickel (II) chloride ($NiCl_2 \bullet 6H_2O$) are added to the solution, and the mixture is stirred for 6 days using a magnetic stirrer [27]. The resulting solution is then dialyzed and stored at 4°C. To determine TC with NiNCs, the optimum conditions require adding 100 μL of 100 mM Eu^{3+} salt europium acetate and 50 μL of NiNCs to a pH 7 buffer solution [27]. The volume of TC and (oxytetracycline) OTC solution used should be 500 μL [27]. In addition to that, reaction time is 10 min before the measurement of fluorescent intensity.

The excitation wavelength required to detect TC and OTC using this detection agent is 365 nm [27]. Figure 3 shows the results obtained from detecting TC and OTC using this agent. The graphs depict 2 emission spectra peaks, where the first peak is from NiNCs and the second

peak is from the connection between Eu^{3+} and TC or OTC [27]. The principle of operation of the detection agent is based on the fact that the Eu^{3+} ions attach to surface of NiNCs, forming an organometallic compound between Eu^{3+} and TC. The linear range and LOD point calculated by ratiometric method mention in Table 1.

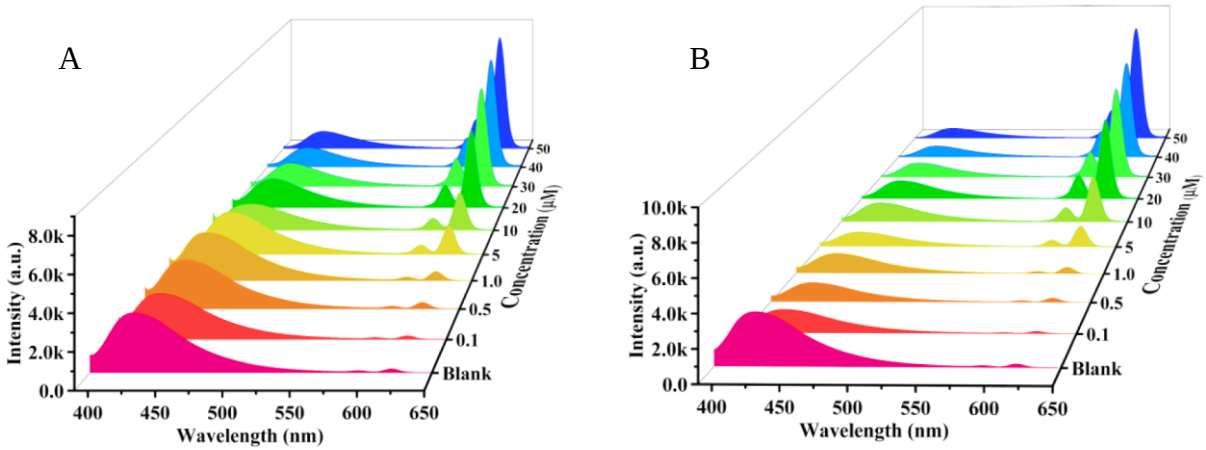

Figure 3. Graph of fluorescent emission of NiNCs with Eu^{3+} with different concentration of A) TC and B) OTC. Source: Adapted with permission from ref. 27. Copyright 2023 Elsevier B.V.

The peak enhancement mechanism at 620 nm is due to the "antenna effect" of TC transferring photon energy to the Eu^{3+} ions and the metal enhancement fluorescent effect of NiNCs [27]. When TC attaches to the agent, it increases the radius of NiNCs-Eu^{3+}. While the emission peak of NiNCs is quenched by TC. This is because the absorption spectra of TC and OTC overlap with the excitation spectra of NiNCs, resulting in the inner filter effect [27].

According to the selectivity test result, NiNCs mixed with Eu^{3+} have a greater intensity emission of OTC than TC (figure 4) [27]. However, it is still selective and determine TC group antibiotic: TC, OTC and chlortetracycline (lower intensity than TC and OTC).

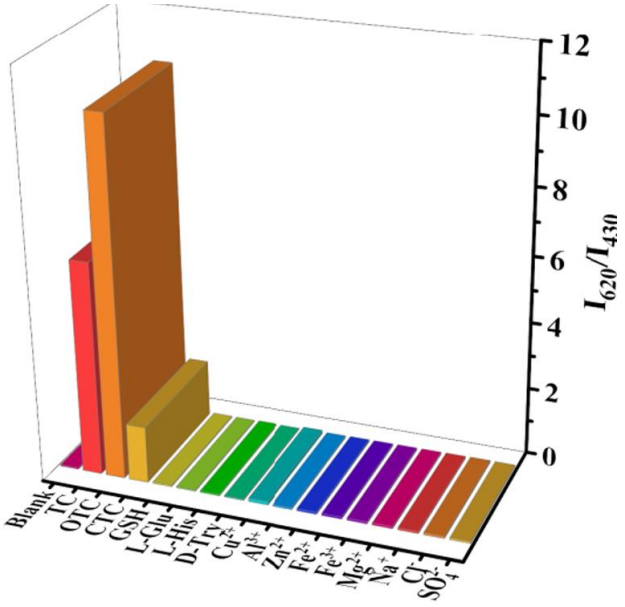

Figure 4. The graph of selectivity test of NiNCs mixed with Eu^{3+} on emission spectra response with different solutions. Source: Adapted with permission from ref. 27. Copyright 2023 Elsevier B.V.

Where: GSH – glutathione, Glu – glucose, His – histidine, Try – tryptophan.

One more detection agent based on gold (Au) nanoclusters (AuNCs) with organic substance [28]. The preparation steps include mixing and stirring two solutions of $HAuCl_4$ and bovine serum albumin (BSA) at 37°C [28]. After two minutes, NaOH is added and the mixed solution is kept at 37°C, resulting in AuNCs@BSA [28]. The same preparation step is applied for AuNCs with His and amino acids of DNA_{C12}. Based on the results, the optimum AuNCs are AuNCs@DNA_{C12} because the emission intensity of AuNCs@DNA_{C12} with Eu^{3+} and with TC is higher than that of AuNCs@BSA and AuNCs@His [28]. The next step is to detect TC using this agent. The results show that the optimum condition is achieved with 25 µM Eu^{3+} from europium oxide (Eu_2O_3), 10 µL AuNCs@DNA_{C12}, and a solution media pH 9 [28]. Different concentrations of TC are then added for detection (Figure 5). Also, according to result, the agent is selective only for TC [28]. The linear range and LOD is in Table 1.

The principle of operation is that Eu^{3+} ions are placed on the surface of AuNCs@DNA_{C12}. TC then combines with Eu^{3+} ions, causing light emit red color under the excitation wavelength 375 nm and AuNCs@DNAC12 plays the role of metal enhancement fluorescent effect [28].

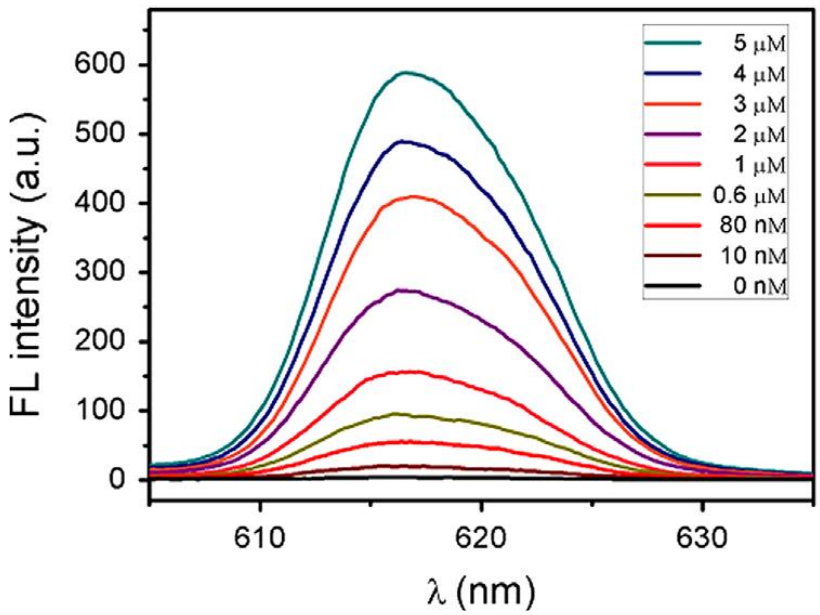

Figure 5. The graph of fluorescent emission spectra peaks of detection agent AuNCs@DNA_{C12} with Eu^{3+} with different concentration of TC. Source: Adapted with permission from ref. 28. Copyright 2013 Elsevier B.V.

The detection agent NH_2-MIL-53(Al) nanosheets doped by Eu^{3+} nanocomposites for TC detection [29]. The preparation of the detecting agent involves several steps. Firstly, the aluminum salt $AlCl_3 \cdot 6H_2O$ is dissolved in ultra-pure water, and 2-amino terephthalic acid (NH_2-H_2BDC) is added to the solution, which is then stirred [29]. Next, a solution of urea with distilled water is added and stirred again. The resulting mixture is poured into an autoclave and placed in a muffle oven at 150°C for 5 hours [29]. Once completed, the solution is washed with ultra-pure water and centrifuged. The product is then transferred to a beaker with 20 mL dimethylformamide (DMF) and 20 mL methanol and stirred for an additional day [29]. Finally, the solution is centrifuged and dried to obtain the Eu^{3+} nanocomposites, which are placed on nanosheets through chemical doping [29]. To achieve this, 50 mg of nanosheet is placed in an ethanol solution containing europium chloride ($EuCl_2 \cdot 6H_2O$) for 2 days [29]. Excess and undoped Eu^{3+} ions are removed by washing with ethanol and centrifugation. The final product obtained is Eu^{3+}/NH_2-MIL-53(Al). The optimum conditions for sensing TC are achieved by adding 2 mL of a buffer solution with pH 9, 100 μL of the agent, and then adding TC solution of different concentrations and waiting 1 min for reaction to detect a linear range of detection [29]. The linear range of detection and LOD point of the agent are listed in Table 1. The calculation of the linear range is ratiometric due to the mechanism of TC detection by the agent. The intensity of the emission spectra peaks of NH_2-MIL-53(Al) decreases with increasing TC concentration, while the intensity of the Eu^{3+} ion part of the agent increases proportionally with the increasing concentration of TC (Figure 6) [29]. The mechanism for detecting TC by

Eu^{3+}/NH_2-MIL-53(Al) involves the fluorescence resonance energy transfer effect [29]. This is due to the overlap of two spectra, which the absorption spectra of TC-Eu^{3+}/NH_2-MIL-53(Al) and the fluorescent spectra of NH_2-MIL-53(Al). The NH_2-MIL-53(Al) acts as the donor of energy and is quenched due to the transfer of energy to the TC-Eu^{3+}/NH_2-MIL-53(Al) energy acceptor complex [29].

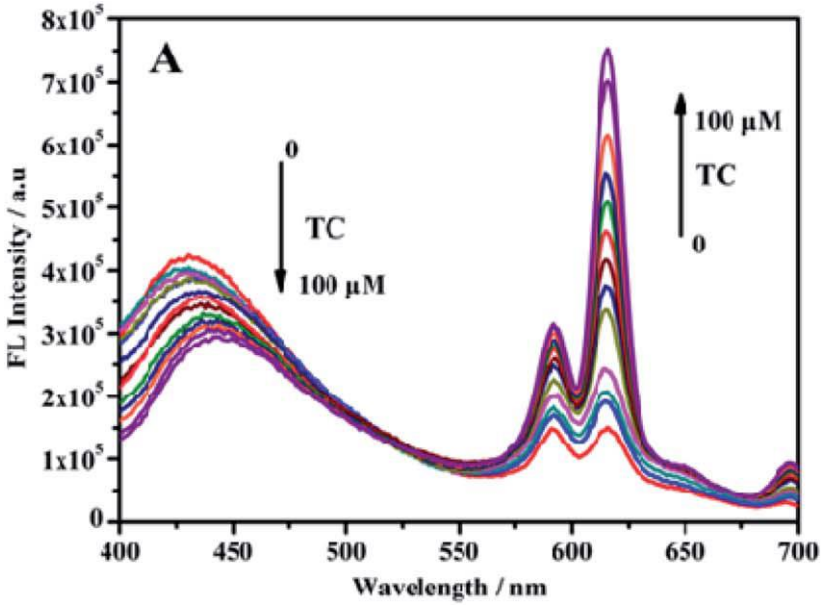

Figure 6. Graph of fluorescent emission spectra peaks of detection TC by detection agent Eu^{3+}/NH_2-MIL-53(Al). Source: Adapted with permission from ref. 29. Copyright 2021 Royal Society of Chemistry.

The excitation wavelength for nanosheets is 339 nm to detect TC [29]. Based on the results of the selectivity test, which involved testing the agent's response to various compounds, it was found that the agent is effective and selective in producing an intensive emission response only in the presence of TC [29]. The selectivity of the agent for TC was confirmed by its minimal response to other organic substance and metal ions, highlighting the suitability of the agent for the specific detection of TC.

Another detection agent of TC is carbon dots (CDs). The preparation of Europium carbon dots (CDs-Eu^{3+}) involves a two-step process. In the first step, CDs are synthesized by dissolving citric acid and cyclen in 10 mL of ultra-pure water in a beaker, which is then placed in a microwave and heated for 5 minutes until it solidifies [30]. In the second step, complex CDs- Eu^{3+} are formed by dissolving the solid product in ultra-pure water and mixing it with 60 μL of europium nitrate ($Eu(NO_3)_3$) [30]. The resulting solution is stirred for 5 hours at 300 rpm to ensure complete mixing and formation of the CDs-Eu^{3+} complex [30]. The quantum yield is 27.6% [30]. The agent can sense TC optimally under solution media pH 9, 1 min. for reaction between agent and TC, and excitation wavelength 350 nm [30]. Figure 7 shows that the blue

emission in the range of 400-520 nm from CDs remains stable during TC detection. The second emission peak at 616 nm is from Eu^{3+} bound to TC.

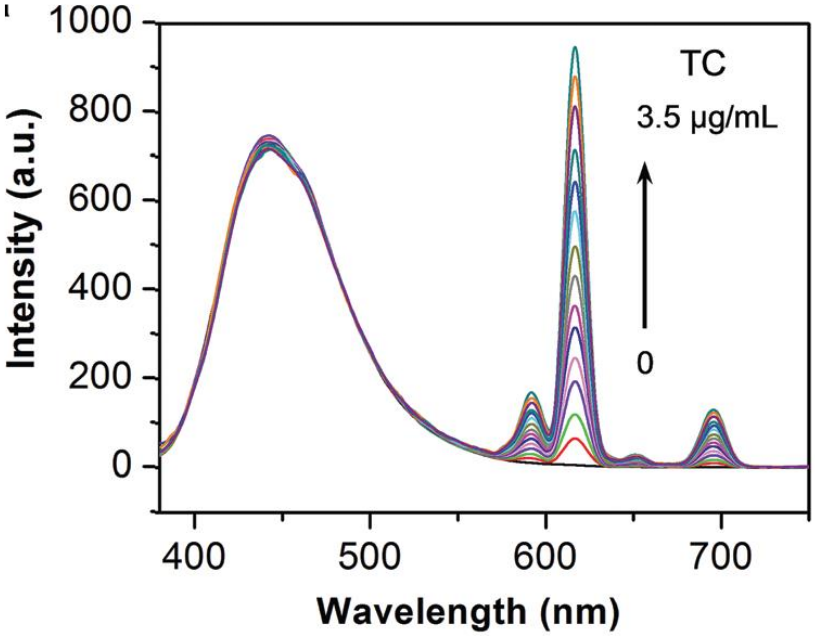

Figure 7. Graph of fluorescent emission spectra peaks of detection TC by detection agent CDs-Eu^{3+}. Source: Adapted with permission from ref. 30. Copyright 2018 Royal Society of Chemistry.

Therefore, the linear range and LOD point are calculated using the ratiometric method (Table 1). As the concentration of TC increases, the Eu^{3+} emission peak also increases, and the color changes from blue to red [30]. The mechanism of TC detection involves Eu^{3+} located on the sphere of CDs forming a complex with TC. TC acts as an antenna to capture photons and enhance the intensity of the fluorescent emission peak of Eu^{3+} [30]. The selectivity test was conducted to evaluate the agent's selectivity towards other antibiotics. Based on the results, the agent showed selectivity towards TC [30].

Another detection agent is Eu doped carbon quantum dots (Eu-CQDs). The process for preparing Eu-CQDs involves the carbonization method [31]. First, two solid substances, citric acid (CA) and europium nitrate hexahydrate ($Eu(NO_3)_3*6H_2O$), are mixed in a round-bottom flask. The flask is then placed in an oil bath and heated at 200°C for 5 minutes [31]. Once cooled, the resulting product is dissolved in water and filtered through a 0.22 μm filter. The product is then subjected to a second filtration process where it is dialyzed through a cellulose ester membrane for 24 hours [31]. In the final step, the product is freeze-dried. According to characterization result, the average size of Eu-CQDs is 3.5 nm and quantum yield is 4.7% [31]. The results show that the emission intensity of Eu-CQDs was quenched in the presence of TC, as illustrated in Figure 8 [31]. The emission of Eu-CQDs is inversely proportional to the concentration of TC, and the excitation wavelength used for sensing TC with this agent is 350

nm [31]. The highest peak of Eu-CQDs was observed at 465 nm [31]. The linear range can be calculated by dividing the initial intensity without TC by the intensity measured in the presence of TC, as shown in Table 1.

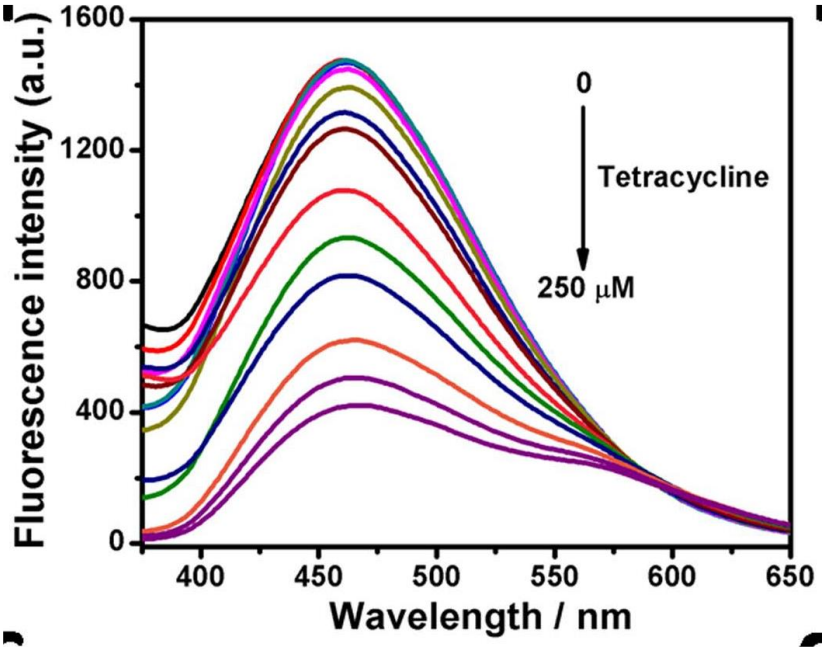

Figure 8. Graph of fluorescent emission spectra peaks of detection TC by detection agent Eu-CQD. Source: Adapted with permission from ref. 31. Copyright 2017 IOP Publishing Ltd.

The quenching effect observed on Eu-CQDs with TC can be attributed to the inner filter effect [31]. This is because the UV-vis spectrometer results indicated no formation of a TC complex with Eu-CQDs, the Raman spectra showed no change in the crystalline structure of Eu-CQDs in the presence of TC, and the fluorescent lifetime remained stable both with and without TC [31].

The detection agent based on carbon dots is CDs with Au nanoclusters nanocomposite [32]. To obtain CDs-AuNCs, CDs were first prepared and then combined with AuNCs. The CDs were prepared using the hydrothermal method in an autoclave at 180°C for 4 hours [32]. The precursor for the CDs was 3-aminophenyl boronic acid, which was dissolved in ultra-pure water and then NaOH was added [32]. The resulting product was filtered through a 0.22 μm membrane filter and subjected to dialysis for 24 hours. The CDs were stored in a dark place at 4°C. To prepare the CDs-AuNCs, the prepared CDs were mixed and stirred with ovalbumin (OVA) for 1 hour [32]. After stirring, $HAuCl_4$ was added to the solution, and the solution was adjusted to pH 9 using NaOH. The solution was then heated to 98°C and stirred for 1 hour [32]. The resulting product was filtered through dialysis for 24 hours and stored in a dark place at 4°C [32]. The optimal conditions for determining TC require 70 μL of the agent, 920 μL of buffer solution with pH 8.5, and 10 μL of TC at different concentrations and time for reaction

between agent and TC is 7 min [32]. The excitation wavelength should be set to 360 nm [32]. The result of interaction CDs-AuNCs with TC shown in Figure 9.

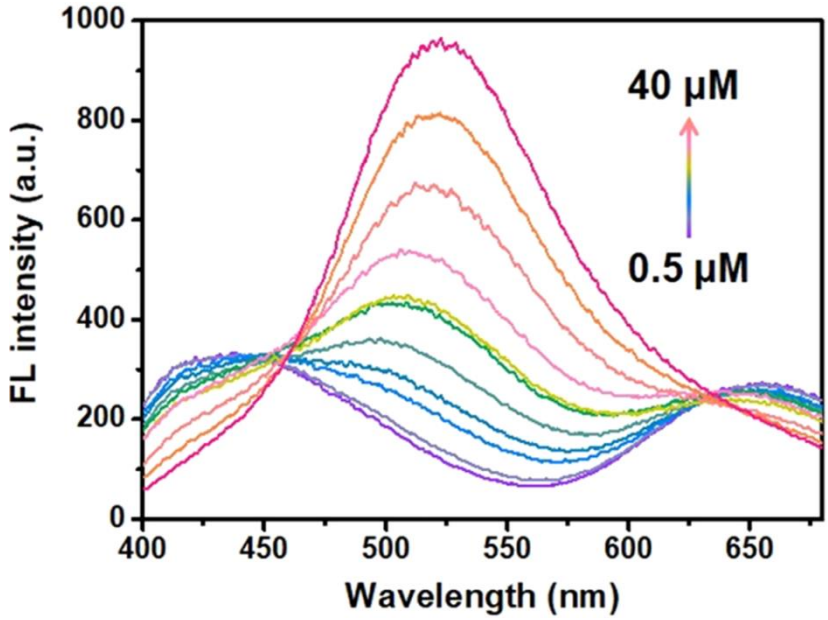

Figure 9. Graph of fluorescent emission spectra peaks of detection TC by detection agent CDs-AuNCs. Source: Adapted with permission from ref. 32. Copyright 2023 Elsevier B.V.

The mechanism of working CDs-AuNCs is that emission peak at 423 nm from CDs and 660 nm from AuNCs quenched by addition of TC [32]. In the same time, TC connects with OVA and by adding TC new intensity emission peak at 520 nm appeared. TC connects with OVA because of intermolecular connection [32]. The emission peak at 423 nm from CDs is quenched due to the inner filter effect. The fluorescent lifetime of CDs in the presence and absence of TC does not show a significant change [32]. Therefore, the explanation for the quenching of the 423 nm emission peak is the inner filter effect [32]. Additionally, the emission peak at 660 nm is quenched due to the disconnection between Au and sulfur (S) in the AuNCs when TC connects with OVA [32].

Another example of detection agent based on CDs. It is cupper (Cu) doped carbon dots (Cu-CDs) [33]. The method for preparing Cu-CDs was achieved through hydrothermal synthesis, using L-tryptophan as the precursor for CDs [33]. After synthesis, the product was filtered using a membrane filter and then underwent dialysis. Finally, the resulting product was freeze-dried and obtained in a solid form [33]. And the quantum yield is 9.5% [33]. The optimum conditions for sensing OTC using Cu-CDs are achieved by diluting the agent in a buffer solution with a media pH of 4, with a concentration of 0.7 mg/mL [33]. The optimum excitation wavelength is 369 nm [33]. Figure 10 illustrates the results, while the linear range and LOD for OTC and TC are presented in Table 1.

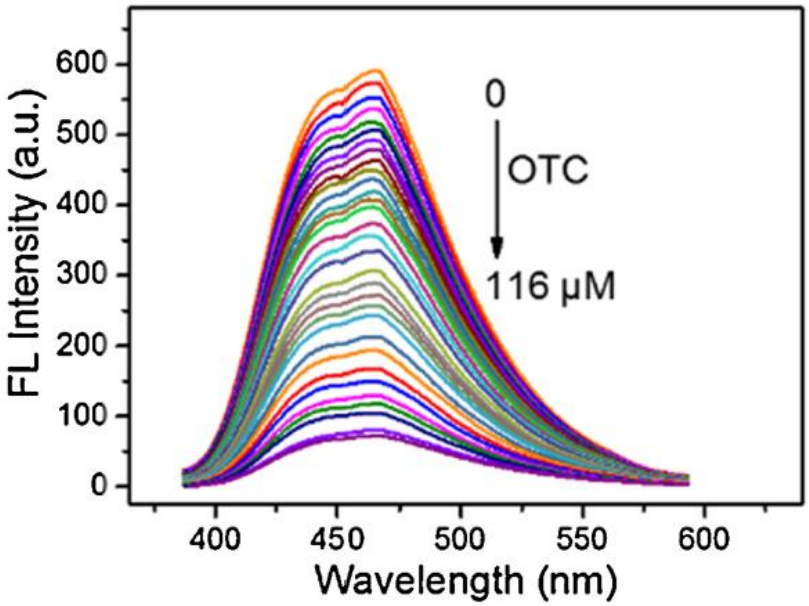

Figure 10. Graph of fluorescent emission spectra peaks of detection OTC by detection agent Cu-CDs. Source: Adapted with permission from ref. 33. Copyright 2020 Elsevier B.V.

This agent detects OTC through the inner filter effect, as the fluorescent lifetime of Cu-CDs does not significantly change in the presence or absence of TC [33]. Additionally, the absorption spectrum results indicate an overlap of the OTC peak with Cu-CDs [33].

One more detection agent based on CDs without doping of ions is red-emitting carbon dots (R-CDs) [34]. The precursors for R-CDs are urea and organic dye resazurin, which are used in the solvothermal method in the autoclave at 200°C for 8 h [34]. The resulting mixture is then filtered with filter paper, subjected to centrifugation to remove bulky particles, and filtered again through a membrane filter [34]. The remaining solution is evaporated, and the resulting solid R-CDs are obtained as the end product. The quantum yield is 58.9% [34]. The optimal conditions for detecting TC using R-CDs are as follows: solution media pH 7.4, agent concentration of 0.06 mg/mL, adding a volume of 100 µL to each sample, and a reaction time of 2 min [34]. The optimum excitation wavelength for the detection agent is 365 nm [34]. The

linear range and LOD point are in Table 1. The graph of emission intensity of detection by R-CDs of different concentration of TC presented in Figure 11. In addition, the result shows of the detection agent provide good selectivity.

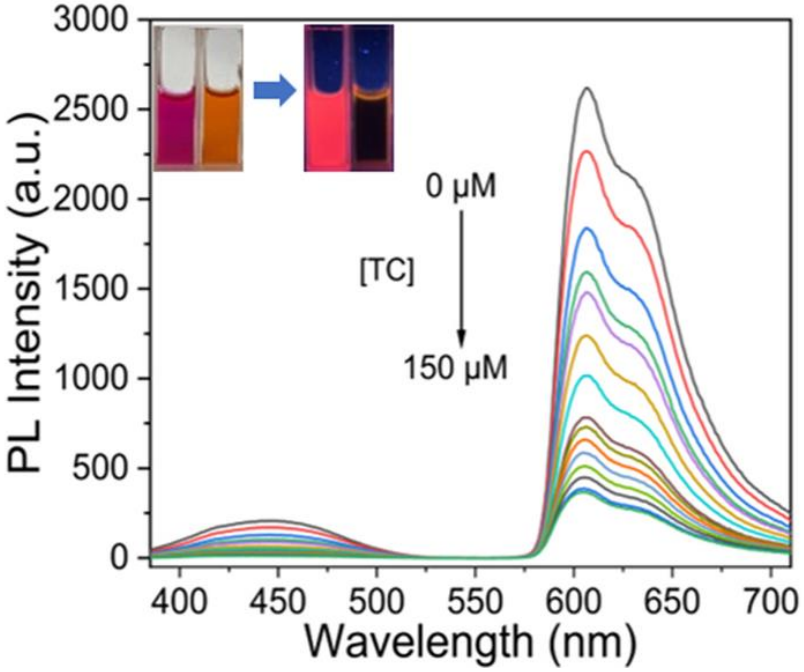

Figure 11. Graph of fluorescent emission spectra peaks of detection OTC by detection agent R-CDs. Source: Adapted with permission from ref. 34. Copyright 2022 Elsevier B.V.

Based on the graph in Figure 11, it can be observed that the emission intensity of R-CDs decreases as the concentration of TC increases [34]. This quenching effect occurs due to the inner filter effect. According to the result of the UV absorption spectrometer, the absorption peak of TC overlaps with the excitation spectra of the agent [34]. Additionally, the fluorescent lifetime of R-CDs remains relatively constant with and without TC [34].

Another example of a detection agent is carbon dots synthesized from carp roe [35]. The synthesis of carbon dots (CDs) from fish roe is accomplished using the hydrothermal method in an autoclave for 12 hours at 200°C [35]. Before the fish roe is ground into a powder, it is dissolved in distilled water. After the hydrothermal reaction, the resulting solution is filtered and centrifuged [35]. The final product is a transparent suspension of CDs with a concentration of 0.1 mg/mL [35]. The quantum yield of CDs is 13.4% [35]. The optimum condition for detecting TC is mixing 1.8 mL of the agent with different concentrations of TC in the tubes [35]. The optimum excitation wavelength is 365 nm [35]. The result of the interaction TC with CDs shows in Figure 12, while the linear range and LOD can be found in Table 1. According to Figure 12, the quenching effect of CDs depends on increasing the concentration of TC [35]. The mechanism of the quenching effect occurs due to the inner filter effect. The study also found that the fluorescent lifetime of CDs remains stable at 3.56 seconds

with and without TC [35]. Further investigation into the UV absorption of TC revealed high peaks at 256 nm and 357 nm, which overlap with the excitation peak of CDs [35]. As a result, TC absorbs the incoming light and quenches the fluorescence of CDs when they are combined.

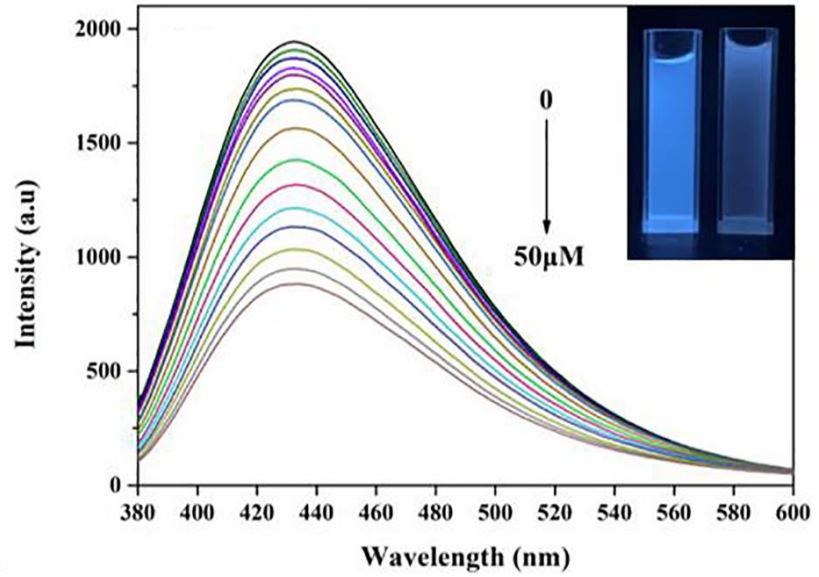

Figure 12. Graph of fluorescent emission spectra peaks of detection OTC by detection agent CDs. Source: Adapted with permission from ref. 35. Copyright 2021 Elsevier B.V.

In addition, the result demonstrates that this detection agent is selective for TC antibiotics [35].

In summary, recent investigations into the detection of TC antibiotics have highlighted the advantages of using the fluorescent method of detection. This is mainly due to the availability of different optical detection agents that can be synthesized from a combination of organic and inorganic substances. Compared to methods such as HPLC, the fluorescent method does not require an operator, making it more convenient and less time-consuming. Moreover, the detection agents used in the fluorescent method can be easily synthesized in a laboratory, providing a good limit of detection and a linear range for the detection of TC. These agents have demonstrated high sensitivity and specificity, making them a reliable and effective tool for detecting TC in various samples. Overall, the literature review suggests that the fluorescent method of detection is a promising approach for the detection of TC antibiotics, offering a range of advantages over other methods. The development of new and more efficient detection agents is likely to further enhance the sensitivity and specificity of this method and expand its application in the field of antibiotic detection.

1.5 Limitations of novel methods of TC detection

While detection agents are effective in detecting tetracycline, they have limitations that make them labor-intensive and time-consuming. In this section, we will analyze the limitations of detection agents as reported in the literature.

The first essential parameter for a detection agent is an easy and efficient production method with minimal synthesis steps. However, some of the detection agents that have been mentioned have limitations when it comes to easy and rapid synthesis. For instance, the detection agents fluorescent Zn-MOF FMOF-5 [26], NiNCs – Eu^{3+} [27], and Eu^{3+}/ NH_2-MIL-53(Al) [29] are time-consuming to prepare. To illustrate, the preparation of FMOF-5 [26] involves first synthesizing Zn-MOF over a period of 20 h. and drying it to obtain a powder. Furthermore, synthesis is not environmentally friendly, and any solid/liquid waste or unreacted reagents must be disposed of properly. Then, the powder needs to be dissolved in ultra-pure water before it can be used for detecting TC [26]. Therefore, FMOF-5 agents have several steps that need to be carried out before they can be used for sensing TC, which can be a limiting factor. One more detection agent that is time-consuming to synthesize is NiNCs-Eu^{3+} [27]. Therefore, to obtain NiNCs, it is necessary to spend 6 days for mixing on a magnetic stirrer [27]. This means that a whole week is spent on the synthesis of nanoclusters, and then they must be stored at a temperature of 4°C. Additionally, to introduce Eu^{3+}, a medium pH of 7 must be created, and only then a solution of tetracycline can be added from above for detection on the fluorescent spectrometer. Another example of a long synthesis is Eu^{3+}/ NH_2-MIL-53(Al) [29]. After the synthesis of NH_2-MIL-53(Al), the nanosheets need to be stirred with a solution of 2 substances, methanol, and DMF, for a day [29]. Then, it takes 2 days to deposit Eu^{3+} ions on the nanosheets. Therefore, a total of 3 days is required to synthesize Eu^{3+}/ NH_2-MIL-53(Al) [29].

Another important aspect to consider is the choice of precursors for the detection agent, which can affect the cost and reproducibility of results. For example, the detection agents AuNCs@DNA_{C12} [28] and CDs-AuNCs [32] both use the expensive gold salt $HAuCl_4$ in their preparation. Another detection agent, CDs synthesized from fish roe [35], has limitations due to fish roe as a precursor. The concentration of toxic heavy metals in the roe needs to be checked before use, as it may impact the reproducibility of results. Furthermore, the authors did not mention the freshness requirements or whether the caviar source affects the parameters of the synthesis. Another factor that affects the reproducibility of results is the equipment used. For instance, in the synthesis of CDs-Eu^{3+} detection agent [30], a domestic microwave was

used. Therefore, before replicating the experiment, it is essential to check the parameters of the microwave to ensure that it still has the same power. Prolonged use of the microwave can cause a decrease in its power output.

Creating optimal conditions for the determination of TC is a limiting factor since it involves adding reagents and takes time. However, there are more rapid methods available, such as adding a detecting agent at different concentrations of TC and adjusting the excitation length on the spectrometer to determine it.

For FMOF-5 agent, it is necessary to maintain the pH in the region from 5 to 7 [26], where the emission intensity of the agent decreases at lower or higher pH levels, affecting its optical detection properties. Similarly, agents such as NiNCs-Eu^{3+} [27] and R-CDs [33] require a permanent pH of 7 and 7.4, respectively, achieved by buffer solution, for effective TC detection. A pH of 9 must be maintained when using AuNCs@DNA_{C12} [28], Eu^{3+}/NH_2-MIL-53(Al) [29], and CDs-Eu^{3+} [30] as detection agents. CDs-AuNCs [32] use a slightly lower pH of 8.5 for TC detection, while a more acidic media pH of 4 is needed for the Cu-CDs [33] detection agent. Furthermore, the detection agents AuNCs@DNA_{C12} [28] and NiNCs-Eu^{3+} [27] require a reaction time of 7 and 10 minutes, respectively, which makes their detection time consuming.

According to the sensitivity test, the authors of the detection agent FMOF-5 do not provide results with other TC group antibiotics such as OTC, CTC, and DOX (Figure 13) [26]. It is unclear whether there is a higher emission response with TC derivative antibiotics or not. This sensitivity test is limited to the optical properties of the FMOF-5 because no results with other antibiotics from the TC group were provided.

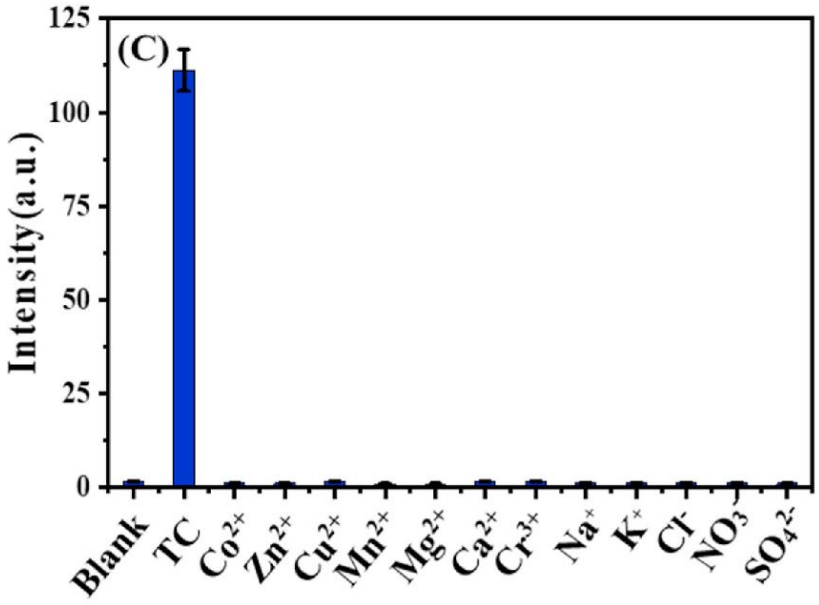

Figure 13. The selectivity test of FMOF-5 detection agent. Source: Adapted with permission from ref. 26. Copyright 2022 Elsevier B.V.

Where: His – histidine, PheA – phenylalanine, Phe – amino acid (authors do not describe), Met – methionine, Trp – tryptophan, GluA – glutamic acid, Lys – lysine, Cys – cysteine, Asp – asparagine, Ser – serine, Gly – glycine, Pro – proline, Tyr – tyrosine.

Comparing the results of the selectivity test of FMOF-5 with the result of selectivity test of detection agent NiNCs-Eu^{3+}, TC exhibited a lower emission response than OTC (Figure 4) [27]. This suggests that this agent is primarily aimed at detecting OTC, with a LOD concentration of 21 nM, while for TC, the LOD concentration is 25 nM [27]. The selectivity test for the detection agent Eu^{3+}/NH_2-MIL-53(Al) [29] presents a similar situation to that of FMOF-5 (Figure 14). The authors only provided a comparison of the emission response with amino acids such as GSH, Glu (glutamic acid), BSA (bovine serum albumin), cysteine (Lys) [29]. Therefore, the selectivity test is not complete enough to consider the detection agent as selective for antibiotics, as there is no comparison with them.

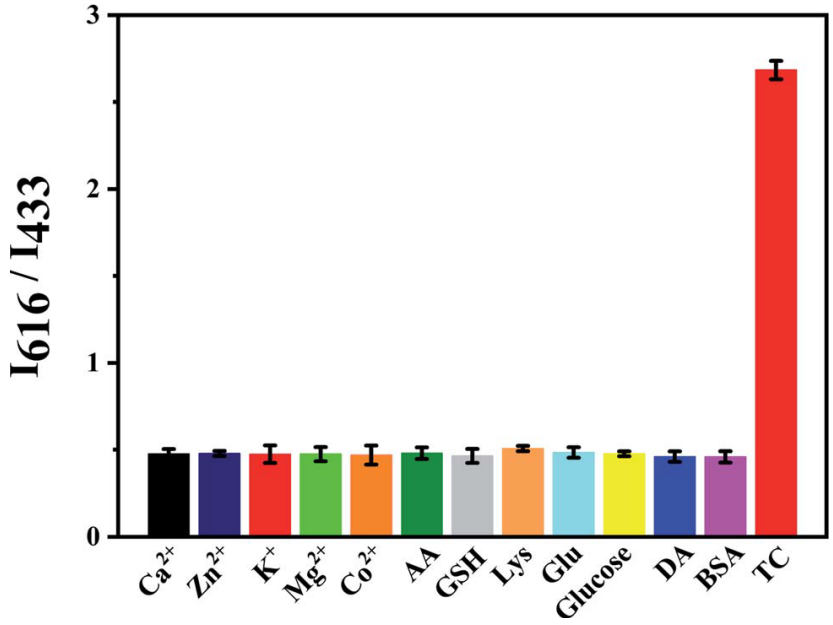

Figure 14. The emission response test of Eu^{3+}/NH_2-MIL-53(Al) on different solutions. Source: Adapted with permission from ref. 29. Copyright 2021 Royal Society of Chemistry.

The next product is the detection agent CDs-Eu^{3+} [30]. The selectivity experiment of CDs-Eu^{3+} reports that the emission response of OTC is slightly lower than the intensity response of TC (Figure 15) [30]. Moreover, the detection agent Eu^{3+}-CDs had a narrow linear detection range from 0 to 3.5 μM [30]. While the expensive synthesized CDs-AuNCs had a wide linear range from 0.5 to 40 μM and a higher LOD point of 0.056 μM [32]. In contrast, the cheaper detection agents Eu-CQDs [31] and Cu-CDs [33] had lower LOD points of 0.3 and 0.17 μM, respectively.

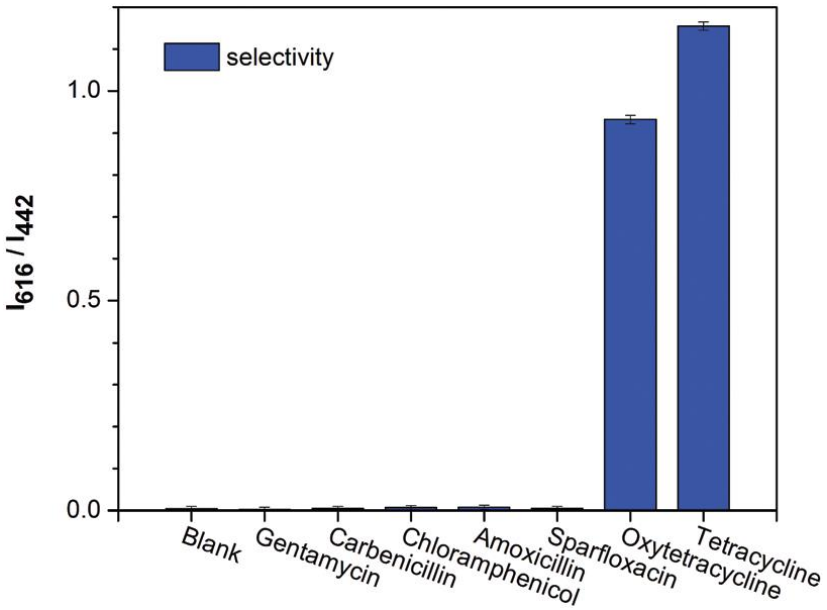

Figure 15. The selectivity test of detection agent CDs-Eu^{3+}. Source: Adapted with permission from ref. 30. Copyright 2018 Royal Society of Chemistry.

Another detection agent study with Eu involves Eu-CQDs [31], which provided a selectivity test but did not compare its results with those of OTC, CTC, and DOX (Figure 16).

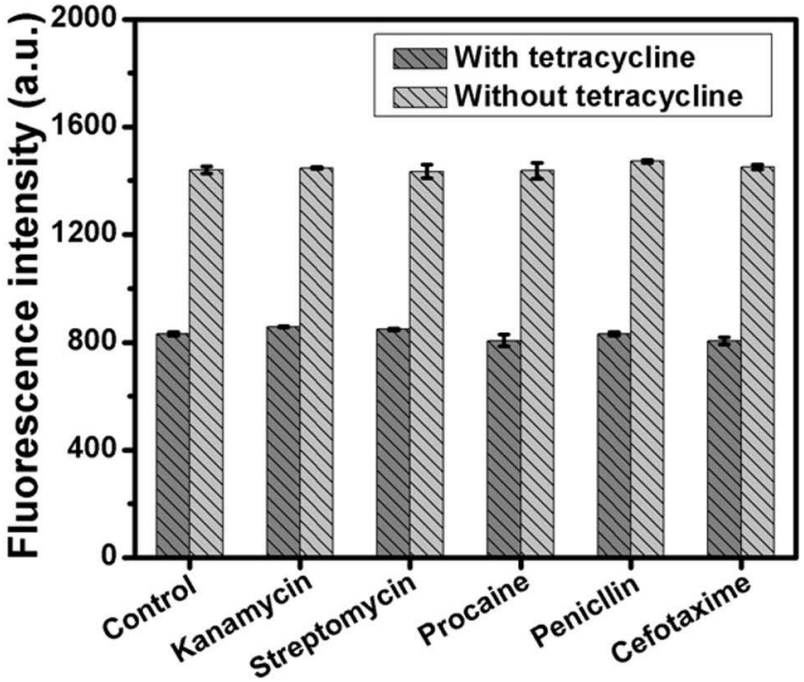

Figure 16. The selectivity test of detection agent Eu-CQDs. Source: Adapted with permission from ref. 31. Copyright 2017 IOP Publishing Ltd.

This review has identified several limitations of detection agents that must be considered when developing new strategies for TC detection. These limitations include time-consuming synthesis, which is not environmentally friendly and generates waste that needs proper disposal (e.g., FMOF-5 [26]). The cost of precursors for the synthesis is another limitation of some detection agents, such as AuNCS@DNA$_{C12}$ [28] and CDs-AuNCs [32]. Some of the detection agents also require a considerable amount of reaction time, such as NiNCs-Eu^{3+} [27] (10 minutes) and AuNCS@DNA$_{C12}$ [28] (7 minutes). The selectivity tests for some agents, including FMOF-5 [26], Eu^{3+}/NH_2-MIL-53(Al) [27], and Eu-CQDs [31], did not compare the intensity response of TC derivatives antibiotics, such as CTC, OTC, and DOX. Additionally, some of the detection agents have a low limit of detection (LOD) for TC detection.

1.6 Objectives of the thesis

1.6.1 Main objectives

Water pollution research has shown that antibiotic pollution is a serious problem that poses a threat to both the ecosystem and human health. TC antibiotics, in particular, have been found to have toxicological effects, such as impaired development of fish and insect embryos, the development of resistance genes, algae growth, and an increase in gut bacteria. Therefore, it is necessary to develop a reliable and quick monitoring of TC concentration in water.

The main aim of this thesis project is to develop highly sensitive and selective detection agent for TC monitoring via easy and environmentally-friendly hydrothermal method. Moreover, extended experimental optimizations were performed to extend linear range of TC detection and determine the selectivity/sensitivity of prepared Eu-CDs.

1.6.2 Thesis structure

The thesis paper is a comprehensive document that comprises 6 main parts and includes 48 pages, 24 figures, and 1 table. The five parts of the thesis include an introduction, methodology, results, conclusion and recommendations, limitation of study, and references.

The introduction part has information about water pollution, TC properties, and toxicology effects on the ecosystem, TC detection with the conventional method (HPLC), a literature review of the novel optical method of TC detection, their limitations, and the objective of the thesis.

The methodology part has 6 subsections which are reagents and equipment, the synthesis process of Eu-CDs, characterization, sensing TC by Eu-CDs, selectivity test and influence of interference ions, and TC sensing in a real sample.

The results part is a report about the result data of characterization of Eu-CDS, TC detection, selectivity test, the influence of metal ions on optical properties of Eu-CDs, and TC detection in a real sample.

The conclusion and recommendations part is about the conclusion of the thesis paper and recommendations for the study.

Limitations of the study report about limitations of the detection agent study.

2. Methodology

2.1.1 Reagents and equipment

High purity reagents were purchased from Sigma-Aldrich and used without purification. Europium nitrate pentahydrate ($Eu(NO_3)_3•5H_2O$), citric acid, melamine, distilled (DI) water, sodium hydroxide (NaOH), sodium chloride (NaCl), tetracycline (TC), ciprofloxacin (CIP), ampicillin (AMP), streptomycin sulfate (STM), erythromycin (ERY), doxycycline monohydrate (DOX), chlortetracycline hydrochloride (CTC), amoxicillin trihydrate (AX), chloramphenicol (CHL), oxytetracycline (OTC), kanamycin (KAN), lincomycin (LIN).

2.1.2 Synthesis process of Eu-CDs

The Eu-CDs were synthesized by one-step hydrothermal method. Firstly, 0.2 g of citric acid, 0.1 g of melamine, and 21.4 mg (0.001 mol) of $Eu(NO_3)_3•5H_2O$ were accurately weighed and transferred into a Teflon liner. Then, 10 mL of DI water was added to the liner. The Teflon liner was inserted into a stainless steel autoclave, which was heated in a muffle oven at 200°C for 12 h. After cooling to room temperature, the prepared solution was filtered through a 0.22 μm membrane filter and dialysis for 24 h to eliminate unreacted substances. The resulting Eu-CDs were stored in a tube at room temperature. In addition, for the characterization of Eu-CDs, the prepared agent was freeze-dried and collected as powder.

2.1.3 Characterization

The excitation and emission depending spectra were measured on fluorescent spectrometer (Shimadzu RF-6000 Spectro fluorophotometer). X-ray photoelectron spectroscopy (XPS) data of Eu^{3+}-CDs were obtained from Omicron MultiProbe XPS instrument. The Fourier-transform infrared (FTIR) spectroscopy data of Eu^{3+}-CDs were measured using the Nicolet iS10 FT-IR Spectrometer equipment.

2.1.4 Sensing TC by Eu-CDs

The detection agent senses TC in a plastic cuvette designed for fluorescence spectrometry, with a total volume of 2 mL. The solution used in the experiment contained different concentrations of TC, ranging from 10 nM to 15 μM, and 100 μL of Eu-CDs. 1 mM of TC solution was prepared by dissolving 88.8 mg TC powder in 30 mL of ethanol and adding 170 mL of DI water. Then this concentration was diluted with DI water to obtain 10 nM, 25

nM, 50 nM, 0.1 μM, 0.3 μM, 0.5 μM, 1 μM, 3 μM, 5 μM, 7 μM, 10 μM, 12 μM and 15 μM. The excitation wavelength for TC detection was 390 nm.

2.1.5 Selectivity test and influence of interference ions

The selectivity test was performed using a concentration of 3 μM, which was prepared from antibiotic powders such as ciprofloxacin (CIP), ampicillin (AMP), streptomycin sulfate (STM), erythromycin (ERY), doxycycline monohydrate (DOX), chlortetracycline hydrochloride (CTC), amoxicillin trihydrate (AX), chloramphenicol (CHL), oxytetracycline (OTC), kanamycin (KAN), lincomycin (LIN). To a cuvette, 2 mL of antibiotic solution and 100 μL of Eu-CDs were added. The data were then measured on a spectrophotometer with an excitation wavelength of 390 nm. Based on the data, a bar chart was generated. In addition, a blank measurement was taken, which consisted of 2 mL of DI water and 100 μL of Eu-CDs with the same concentration of TC as antibiotics, along with the detection agent.

The influence of ionic strength on TC detection by Eu-CDs was the next experiment. The experiment involved adding 1.9 mL of a 50, 100, or 200 ppm NaCl solution to each cuvette, followed by the addition of 100 μL of 20 μM TC and 100 μL of Eu-CDs.

The investigation of the effect of pH on the detection of TC by the agent was the subsequent experiment. The pH solutions, ranging between 5 to 9, were prepared with diluted NaOH and HCl solutions and DI water. Next, 1.9 mL of each pH solution was transferred to a cuvette, followed by the addition of 100 μL of 20 μM TC and 100 μL of Eu-CDs. The resulting mixture was then measured by using fluorescent spectrometer.

3. Results

3.1.1 Characterization

The Eu-CDs were obtained through a one-step hydrothermal synthesis process that lasted for 12 hours at 200°C. The resulting product was then filtered through a 0.22 μm membrane filter and dialyzed for 24 hours to eliminate unreacted substances. After the filtration process, the agent was freeze-dried for 24 hours for characterization.

The XPS and FT-IR data report the detection and analysis of the elemental composition and functional groups present in Eu-CDs. Based on graph A in Figure 17, the XPS measurement of Eu-CDs shows base peaks at 285.3, 440.0, 532.7, and 1145.6 eV for C1s, N1s, O1s, and Eu3d, respectively [30, 31, 35]. The C1s spectrum (Figure 17, B) reveals peaks at 285.2 eV for C-C and C=C, 286.5 eV for C-O and C-N, and 288.87 eV for C=O and C=N [30, 31, 35, 36, 37]. Similarly, the O1s spectrum (Figure 17, C) exhibits two peaks at 532.02 and 533.57 eV for C-O and C=O, respectively [38].

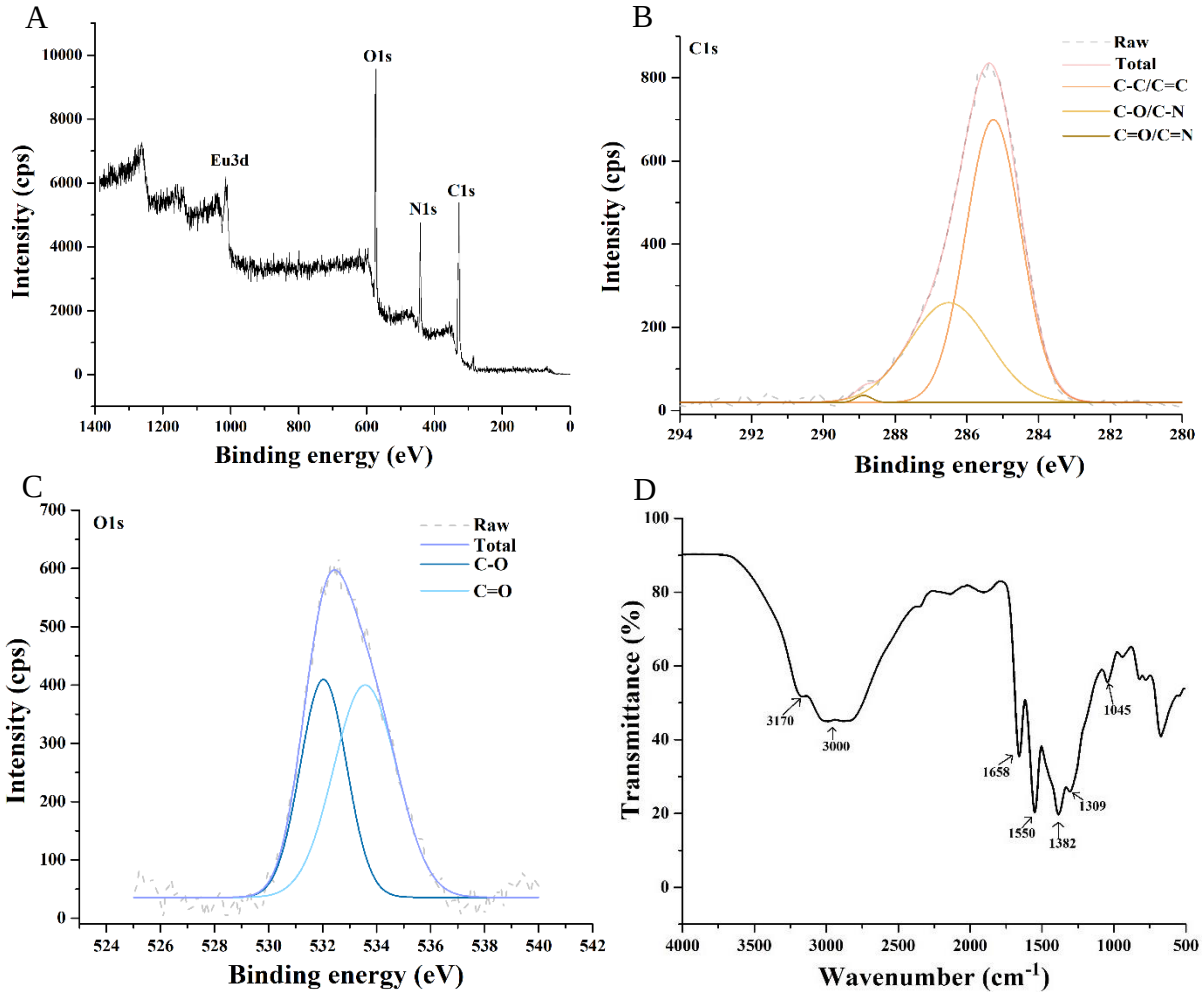

Figure 17. The graph of A) XPS survey spectra of Eu-CDs, B) C1s peaks and fitting curves, C) O1s peaks and fitting curves, D) FT-IR spectra of Eu-CDs

According to FT-IR spectroscopy data (Figure 17, D), the functional groups of Eu-CDs were identified as follows: a stretch vibration of N-H at 3170 cm^{-1} and a bend vibration at 1550 cm^{-1} [35], a broad and intensive peak at 3000 cm^{-1} representing the O-H functional group [31], characteristic peaks at 1658 cm^{-1} and 1045 cm^{-1} indicating the presence of C=O and C-O carboxylic acid functional groups [35], and intensive peaks of 1382 and 1309 cm^{-1} corresponding to the C-N functional group [31]. Therefore, the FT-IR spectra clearly showed the existing of functional groups of both melamine and citric acid. The quantum yield of Eu-CDs was 14.4%.

3.1.2 Sensing TC by Eu-CDs

The fluorescence spectrometer was used in the next experiment to determine the excitation-dependent emission spectrum and the optimum excitation wavelength for agent and TC sensing. The excitation wavelength was changed from 320 nm to 380 nm in order to identify the optimum excitation wavelength of Eu-CDs. The intensity of the emission peaks was measured, and the results are shown in Figure 18 A. The most intense emission peak was found to occur at an excitation wavelength of 340 nm. The excitation peak of the agent was identified at 341 nm. We prepared the controlled concentration of TC and measured the fluorescent emission of a controlled concentration of TC (1 μM) and volume (2 mL) using 100 μL of the agent at different excitation wavelengths, we obtained different results in terms of the intensity peaks of the fluorescent emission of pure Eu-CDs. The data is shown in Figure 18 B. The graph in Figure 18 B clearly indicates that the emission intensity at an excitation wavelength of 340 nm was lower than at 390 nm. As a result, the optimum excitation wavelength for Eu-CDs in the detection of TC was found to be 390 nm.

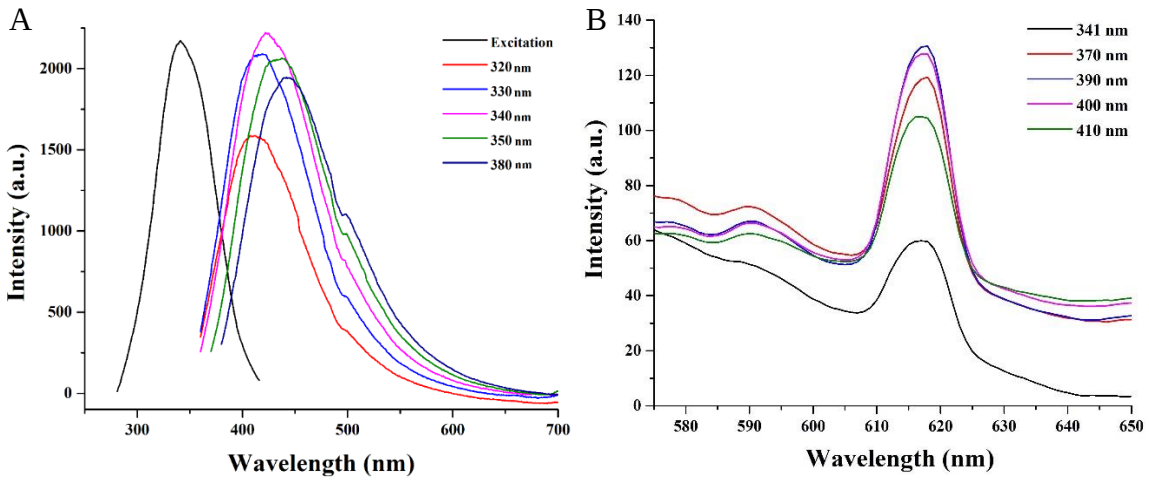

Figure 18. A) The graph of excitation spectra and emission spectra peaks at different excitation wavelengths of Eu-CDs, B) The graph of emission spectra of Eu peak at the different excitation wavelengths of Eu-CDs with TC

The influence of excitation wavelengths 340 and 390 nm on the ability to detect TC was examined, and measurements of TC concentrations ranging from 0.01 to 15 µM were taken using these wavelengths. The results showed that the sensitivity of Eu-CDs varied depending on the excitation wavelength, as shown in Figure 19. The peak associated with Eu was demonstrated in Graph B of Figure 19 with the highest intensity observed when excited at 390 nm. In contrast, graph A showed a more intense peak of CDs compared to the Eu peak, which affected the sensitivity of TC detection by the agent.

The result (Figure 19, B) showed that the emission intensity of Eu-CDs at 615 nm increased as the concentration of TC increased. Furthermore, the color of the emitted light changed from green-blue to red with an increasing concentration of TC (Figure 20).

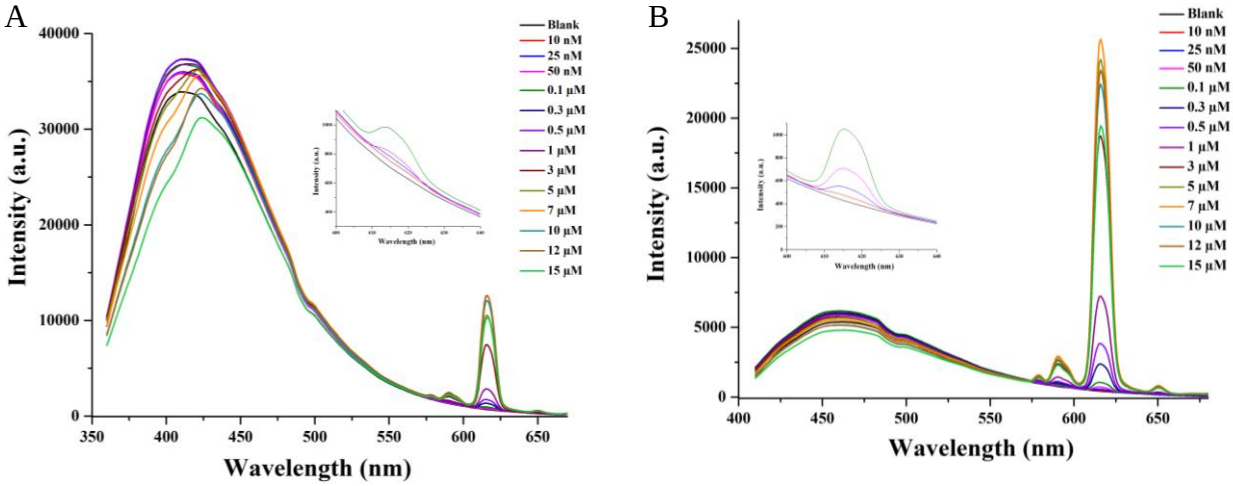

Figure 19. The graph of fluorescent emission of Eu-CDs with different concentration TC A) 340 nm, B) 390 nm

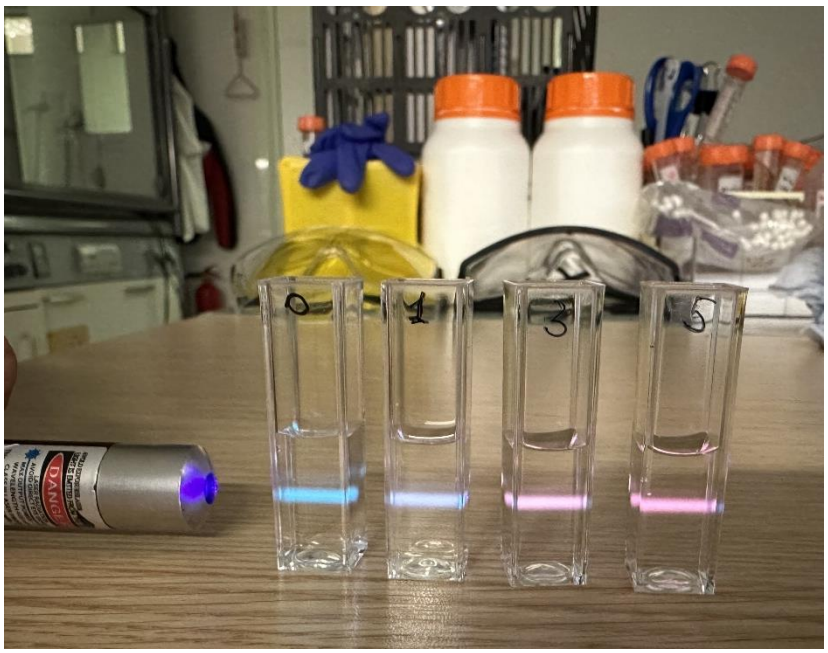

Figure 20. The image of the emitting light changes from green-blue to red with increasing concentration TC

Based on the intensity data at 615 nm from Graph B in Figure 19, we plotted a graph (Figure 21, A) showing the linear relationship between I/I_0 and different concentrations of tetracycline, where I is the intensity of associated with Eu $^5D_0 \rightarrow {}^7F_2$ transition at 615 nm vs. TC concentration, and I_0 is the intensity of the blank (Eu-CDs and no TC). Each sample was taken 2 times. The graph (Figure 21, A) indicates that a linear range is maintained from 0.01 to 5 μM, with an R^2 value of 0.97. Therefore, we established the linear range of detection for TC by Eu-CDs to be from 0.01 to 5 μM, and the limit of detection (LOD) was calculated to be 0.0065 μM or 6.5 nM (Figure 21, B) which is better than other [27, 29, 30, 31, 32, 33, 34, 35] research work.

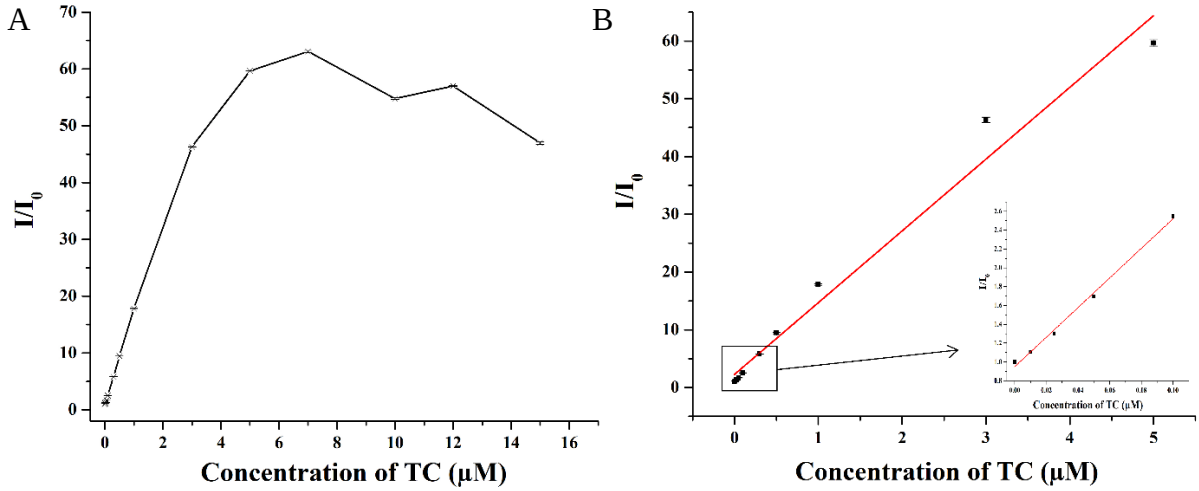

Figure 21. A) The linear intensity response of concentration TC, B) The linear range of detection TC by Eu-CDs

After a TC concentration of 5 μM from Graph A (Figure 21), a saturation of the detecting agent with tetracycline occurs. Therefore, no linear dependence is observed.

The increase in emission intensity with increasing TC concentration can be explained by the "antenna" effect of TC on Eu^{3+} ions. TC forms a complex with Eu [39] and acts as an antenna that absorbs photons from the excitation wavelength 390 nm. The absorbed energy is then transferred to Eu, resulting in an increase in emission intensity at 615 nm wavelength. At the same time 390 nm is optimal excitation for level $^5L_6$ of Eu^{3+} ion (Figure 22 B) [40]. Excitation occurs within the europium atom, causing the electron transition from the $^5D_0 \rightarrow {}^7F_2$ level, resulting in emission of red photons [30, 40]. While the other research [30, 31] of CDs with Eu^{3+} ion used excitation wavelength 350 nm.

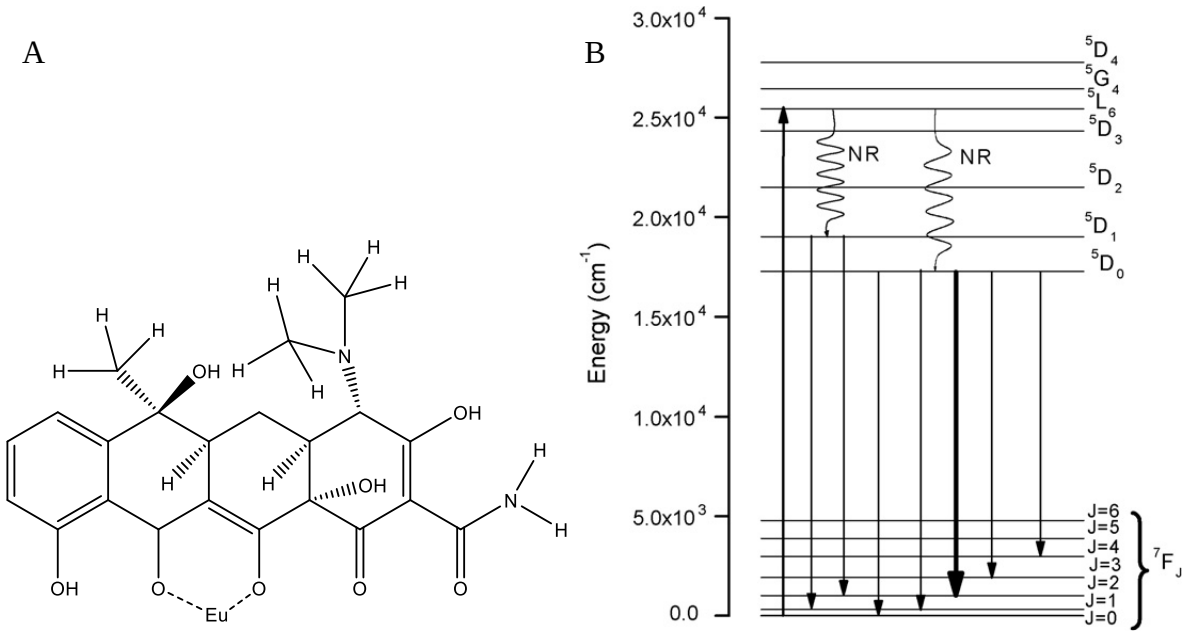

Figure 22. A) The structure of the complex EuTC, B) The energy level diagram of Eu^{3+}. Source: Adapted with permission from ref. 40. Copyright 2009 Elsevier B.V.

3.1.3 Selectivity test and influence of interference ions

A selectivity test was conducted using other antibiotics at a concentration of 3 µM and an excitation wavelength of 390 nm. The results showed that Eu-CDs exhibited selectivity towards TC mainly and had lower intensity responses from other TC derivatives like OTC, CTC, and DOX (Figure 23).

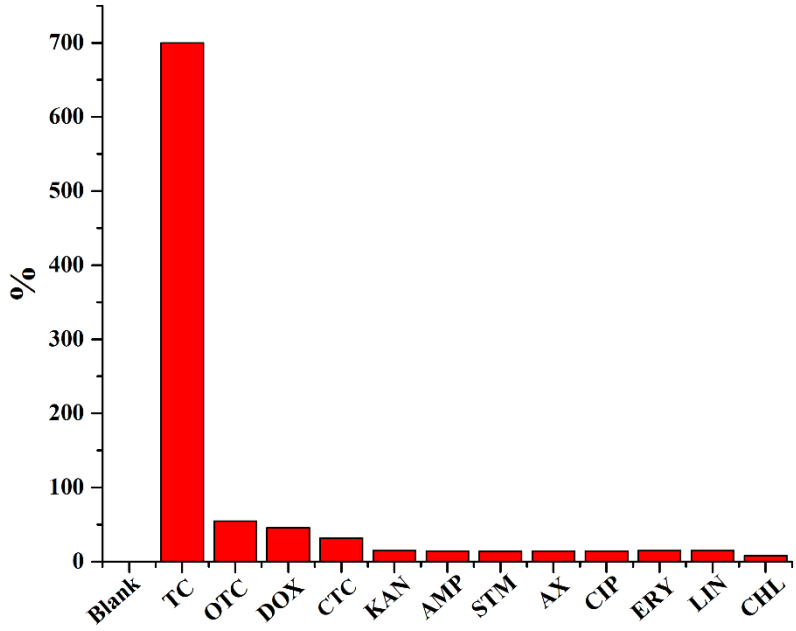

Figure 23. The selectivity test of Eu-CDs with different antibiotics

Next, we investigate the impact of ionic strength by NaCl solution and different pH media on the detection TC by Eu-CDs. The results showed that the detection of TC (at a

controlled concentration of 1 μM) was possible even in the presence of NaCl at concentrations of 50, 100, and 200 ppm (Figure 24, A). This is because, in comparison with the blank sample (Eu-CDs with 1μM TC), the presence of NaCl did not significantly affect the emission intensity of the agent and binding ability of formation EuTC complex. The next graph shows the influence of pH on the detection properties of Eu-CDs. It reveals that the highest emission intensity for detecting TC was observed at pH 7, while other pH conditions resulted in a decrease in the Eu peak intensity with TC (Figure 24, B). Hanse, it was concluded the optimum pH for measurement should be arranged around 7.

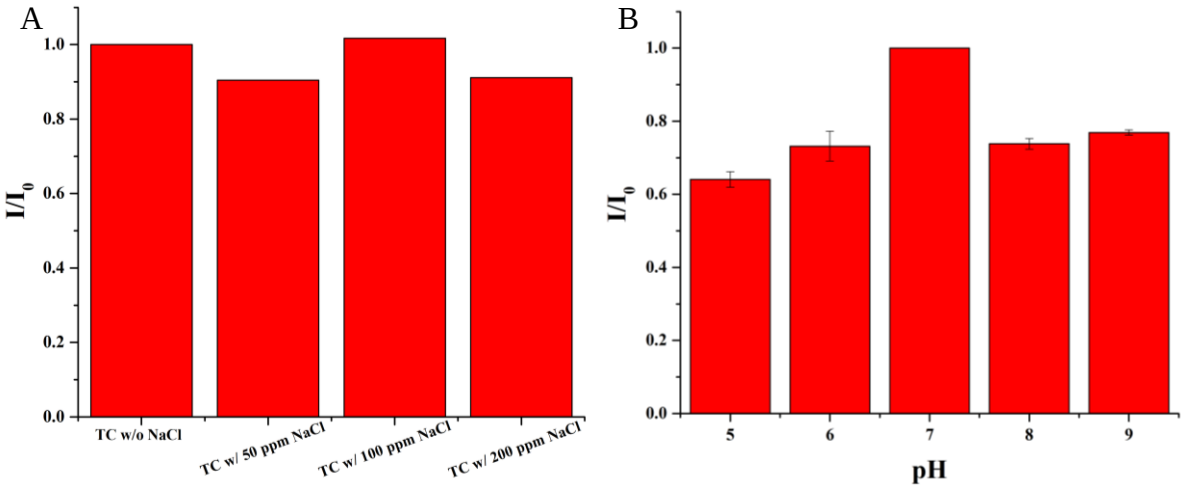

Figure 24. A) The bar graph of influence of NaCl on the detection TC by Eu-CDs, B) The bar graph of influence of pH on the detection TC by Eu-CDs

4. Conclusions and Recommendations

Agricultural activities, especially animal farming, are the main cause of TC contamination of near water resources. TC is widely used for the treatment of animals and as a feed additive in livestock farming. TC contamination has serious toxicological effects on living ecosystems [6, 10, 11, 12] and human health [18,19,20].

Typically, HPLC with the MS method are commonly employed for the determination of TC antibiotic in water samples [21, 22, 23, 24, 25]. However, these methods are time consuming, require costly equipment and trained operator. Therefore, an alternative and cheaper method for the determination of TC is the fluorescent method with a detection agent. To date, novel optical detection agents such as FMOF-5 [26], NiNCs-Eu^{3+} [27], AuNCs@DNA_{C12} [28], Eu^{3+}/NH_2-MIL-53(Al) [29], CDs-Eu^{3+} [30], Eu-CQDs [31], CDs-AuNCs [32], Cu-CDs [33], R-CDs [34], and CDs [35] are proposed. Although, these agents showed effective TC detection, each of them has limitations such as usage of costly precursors for the synthesis, time-consuming synthesis, not being environmentally friendly because of waste generation, extended preparation time, insufficient data in the selectivity tests, and low LOD values. Thus, this thesis proposes a new detection agent that overcomes the limitations of reported agents in the literature.

Eu-CDs was synthesized with one-step hydrothermal synthesis. It was shown that optimum excitation wavelength is 390 nm which is the main novelty of current study, we excited the Eu^{3+} ions directly, while other studies performed excitation of CDs [30, 31]. As a result, we found that the linear range of Eu-CDs was from 0.01 to 5 μM. The LOD point was 6.5 nM, that is higher than the LOD of NiNCs-Eu [27], Eu/NH-MIL-53(Al) [29], CDs-Eu [30], Eu-CQDs [31], CDs-AuNCs [32], Cu-CDs [33], R-CDs [34], and CDs [35]. According to the result of the selectivity test of the agent, the Eu-CDs agent was selective only to TC antibiotic and negligible response to TC derivatives such as OTC, DOX, and CTC were observed. The ionic strength experiment showed that the NaCl solution has an insignificant impact on TC detection, while the optimum pH condition for TC detection was pH 7.

As a recommendation for future research, we will investigate and try to locate water resources from local farms where grazing animals drink. We will compare the results of TC detection by using HPLC with those obtained using from PL spectroscopy.

5. Limitations of study

Several limitations of the study are exist. In particular these limitations are listed below and expected to be addressed in near future.

- The absence of TEM measurement – require to assess the morphology and size distribution of prepared agent.
- The experiment with TC detection in real water samples was not provided.
- Compare TC detection with conventional HPLC.
- The absence of time resolved photoluminescence spectroscopy of the prepared agent.

6. References

1 Liu, Y.; Wang, P.; Gojenko, B.; Yu, J.; Wei, L.; Luo, D.; Xiao, T. A review of water pollution arising from agriculture and mining activities in Central Asia: Facts, causes and effects. *Environ. Pollut.* **2021**, *291*, 118209.

2 Gorlachev, I.; Kharkin, P.; Dyussembayeva, M.; Lukashenko, S. N.; Gluchshenko, G.; Matiyenko, L.; Zheltov, D. A.; Kitamura, A.; Khlebnikov, N. Comparative analysis of water contamination of the Shagan river at the Semipalatinsk test site with heavy metals and artificial radionuclides. *J. Environ. Radioact.* **2020**, *213*, 106110.

3 Zhou, L.; Li, S.; Li, F. Damage and elimination of soil and water antibiotic and heavy metal pollution caused by livestock husbandry. *Environ. Res.* **2022**, *215*, 114188.

4 Pulicharla, R.; Hegde, K.; Brar, S. K.; Surampalli, R. Y. Tetracyclines metal complexation: Significance and fate of mutual existence in the environment. *Environ. Pollut.* **2017**, *221*, 1–14.

5 Rahmani, M.; Álvarez, S. E. N.; Hernández, E. B. The potential use of tetracyclines in neurodegenerative diseases and the role of nano-based drug delivery systems. *Eur. J. Pharm. Sci*. **2022**, *175*, 106237.

6 Scaria, J.; Anupama, K.; Nidheesh, P. V. Tetracyclines in the environment: An overview on the occurrence, fate, toxicity, detection, removal methods, and sludge management. *Sci. Total Environ*. **2021**, *771*, 145291.

7 Dai, Y.; Liu, M.; Li, J.; Yang, S.; Sun, Y.; Sun, Q.; Wang, W.; Lu, L.; Zhang, K.; Xu, J.; Zheng, W.; Hu, Z.; Yang, Y.; Gao, Y.; Liu, Z. A review on pollution situation and treatment methods of tetracycline in groundwater. *Sep. Sci. Technol*. **2020b,** *55*(5), 1005–1021.

8 Xu, L.; Zhang, H.; Xiong, P.; Zhu, Q.; Liao, C.; Jiang, G. Occurrence, fate, and risk assessment of typical tetracycline antibiotics in the aquatic environment: A review. *Sci. Total Environ*. **2021**, *753*, 141975.

9 Daghrir, R.; Drogui, P. Tetracycline antibiotics in the environment: a review. *Environ. Chem. Lett*. **2013**, *11*(3), 209–227.

10 Han, T.; Baoshi, W.; Wu, Z.; Dai, C.; Zhao, J.; Mi, Z.; Yang, L.; Zhang, C.; Miao, X.; Zhou, J.; Li, X.; Sun, Z.; Yang, J.; Zhai, W.; Zheng, F.; Chen, Z.; Zhang, B. Providing a view for toxicity mechanism of tetracycline by analysis of the connections between metabolites and biologic endpoints of wheat. *Ecotoxicol. Environ. Saf*. **2021**, *212*, 111998.

11 Moullan, N.; Mouchiroud, L.; Wang, X.; Ryu, D.; Williams, E. R.; Mottis, A.; Jovaisaite, V.; Frochaux, M.; Quirós, P. M.; Deplancke, B.; Houtkooper, R. H.; Auwerx, J. Tetracyclines

Disturb Mitochondrial Function across Eukaryotic Models: A Call for Caution in Biomedical Research. *Cell Rep*. **2015**, *10*(10), 1681–1691.

12 Leichtweis, J.; Vieira, Y.; Welter, N.; Silvestri, S.; Dotto, G. L.; Carissimi, E. A review of the occurrence, disposal, determination, toxicity and remediation technologies of the tetracycline antibiotic. *Chem. Eng. Res.Des*. **2022**, *160*, 25–40.

13 Chen, H.; Zheng, W.; Shen, X.; Wang, S.; Zhou, X.; Shen, J.; Lu, M. Occurrence, Distribution, and Ecological Risk Assessment of Antibiotics in Different Environmental Media in Anqing, Anhui Province, China. *Int. J. Environ. Res. Public Health*. **2021**, *18*(15), 8112.

14 Han, B.; Yang, F.; Tian, X.; Mu, M.; Zhang, K. Tracking antibiotic resistance gene transfer at all seasons from swine waste to receiving environments. *Ecotoxicol. Environ. Saf*. **2021b**, *219*, 112335.

15 Mo, Y.; Chen, Z.; Leung, W. H.; Leung, A. O. W. Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks. *Environ. Sci. Pollut. Res*. **2017**, *24*(10), 8978–8989.

16 Huang, M.; Zhang, W.; Liu, C.; Hu, H. Fate of trace tetracycline with resistant bacteria and resistance genes in an improved AAO wastewater treatment plant. *Chem. Eng. Res. Des*. **2015**, *93*, 68–74.

17 Morshdy, A. E. M.; Hussein, M.; Mohamed, M. A.; Hamed, E.; El-Murr, A. E.; Darwish, W. S. Tetracycline residues in tilapia and catfish tissue and the effect of different cooking methods on oxytetracycline and doxycycline residues. *JCF*. **2022**, *17*(4), 387–393.

18 Ahn, Y.; Jung, J. Y.; Kweon, O.; Veach, B. T.; Khare, S.; Gokulan, K.; Pineiro, S. A.; Cerniglia, C. E. Impact of Chronic Tetracycline Exposure on Human Intestinal Microbiota in a Continuous Flow Bioreactor Model. *Antibiotics*. **2021**, *10*(8), 886.

19 Jung, J. Y.; Ahn, Y.; Khare, S.; Gokulan, K.; Pineiro, S. A.; Cerniglia, C. E. An in vitro study to assess the impact of tetracycline on the human intestinal microbiome. *Anaerobe*. **2018b**, *49*, 85–94.

20 Gokulan, K.; Cerniglia, C. E.; Thomas, C. R.; Pineiro, S. A.; Khare, S. Effects of residual levels of tetracycline on the barrier functions of human intestinal epithelial cells. *Food Chem. Toxicol*. **2017**, *109*(Pt 1), 253-263.

21 Selvam, A.; Kwok, K.; Chen, Y.; Cheung, A.; Leung, K. S. Y.; Wong, J. W. C. Influence of livestock activities on residue antibiotic levels of rivers in Hong Kong. *Environ. Sci. Pollut. Res*. **2016**, *24* (10), 9058–9066.

22 Li, Y.; Liu, B.; Zhang, X.; Wang, J.; Gao, S. The distribution of veterinary antibiotics in the river system in a livestock-producing region and interactions between different phases. *Environ. Sci. Pollut. Res.* **2016,** *23*(16), 16542–16551.

23 Wang, Z.; Chen, Q.; Zhang, J.; Dong, J.; Yan, H.; Chen, C.; Feng, R. Characterization and source identification of tetracycline antibiotics in the drinking water sources of the lower Yangtze River. *J. Environ. Manage*. **2019**, *244*, 13–22.

24 Zhang, K.; Ruan, R.; Zhang, Z.; Zhi, S. An exhaustive investigation on antibiotics contamination from livestock farms within sensitive reservoir water area: Spatial density, source apportionment and risk assessment. *Sci. Total Environ.* **2022**, *847*, 157688.

25 Huang, X.; Chen, C. Y.; Zeng, Q.; Ding, D.; Gu, J.; Mo, J. Field study on loss of tetracycline antibiotics from manure-applied soil and their risk assessment in regional water environment of Guangzhou, China. *Sci. Total Environ*. **2022**, *827*, 154273.

26 Ameen, S. S. M.; Mohammed, N. M. S.; Omer, K. M. Ultra-small highly fluorescent zinc-based metal organic framework nanodots for ratiometric visual sensing of tetracycline based on aggregation induced emission. *Talanta*. **2023**, *254*, 124178.

27 Wang, Y.; Nie, L.; Hua, Y.; Gong, L.; Qiu, X.; Guo, H. A simple paper-based nickel nanocluster-europium mixed ratio fluorescent probe for rapid visual sensing of tetracyclines. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2023**, *292*, 122431.

28 Yang, X.; Zhu, S.; Dou, Y.; Zhuo, Y.; Luo, Y.; Feng, Y. Novel and remarkable enhanced-fluorescence system based on gold nanoclusters for detection of tetracycline. *Talanta*. **2014b**, *122*, 36–42.

29 Chen, J. M.; Xu, Y.; Li, S.; Xu, F.; Zhang, Q. Ratio fluorescence detection of tetracycline by a Eu^{3+}/NH_2-MIL-53(Al) composite. *RSC Adv.* **2021**, *11*(4), 2397–2404.

30 Shen, Z.; Zhang, C.; Yu, X.; Li, J.; Wang, Z.; Zhang, Z.; Liu, B. Microwave-assisted synthesis of cyclen functional carbon dots to construct a ratiometric fluorescent probe for tetracycline detection. *J. Mat. Chem. C*. **2018**, *6*(36), 9636–9641.

31 Liu, M. Z.; Chen, B.; Yang, T.; Wang, J.; Liu, X.; Huang, C. Z. One-pot carbonization synthesis of europium-doped carbon quantum dots for highly selective detection of tetracycline. *Methods Appl. Fluores*. **2017b**, *5*(1), 015003.

32 Miao, J.; Ji, W.; Yu, J.; Cheng, J.; Huang, Y.; Arabi, M.; Zhou, N.; Li, B.; Zhang, Z.; Chen, L.; Wang, X. A triple-emission ratiometric fluorescence sensor based on carbon dots-Au nanoclusters nanocomposite for detection of tetracycline. *Sens. Actuators B Chem*. **2023**, *384*, 133636.

33 Guo, J.; Lu, W.; Zhang, H.; Meng, Y.; Du, F.; Shuang, S.; Dong, C. Copper doped carbon dots as the multi-functional fluorescent sensing platform for tetracyclines and pH. *Sens. Actuators B Chem*. **2021**, *330*, 129360.

34 Wang, B.; Gu, C.; Jiao, Y.; Gao, Y.; Liu, X.; Guo, J.; Qian, T. Novel preparation of red fluorescent carbon dots for tetracycline sensing and its application in trace determination. *Talanta*. **2022b**, *253*, 123975.

35 Tang, X.; Wang, L.; Ye, H.; Zhao, H.; Zhao, L. Biological matrix-derived carbon quantum dots: Highly selective detection of tetracyclines. *J. Photochem. Photobiol. A.* **2022**, *424*, 113653.

36 Amadio, E.; Cailotto, S.; Campalani, C.; Branzi, L.; Raviola, C.; Ravelli, D.; Cattaruzza, E.; Trave, E.; Benedetti, A.; Selva, M.; Perosa, A. Precursor-Dependent Photocatalytic Activity of Carbon Dots. *Molecules*. **2019**, *25*(1), 101.

37 Ren, J.; Malfatti, L.; Granozzi, G. Citric Acid Derived Carbon Dots, the Challenge of Understanding the Synthesis-Structure Relationship. *C*. **2020**, *7*(1), 2.

38 Komarala, E. P.; Nigam, S.; Aslam, M.; Bahadur, D. In-vitro evaluation of layered double hydroxide–Fe_3O_4 magnetic nanohybrids for thermo-chemotherapy. *New J. Chem*. **2016**, *40*(1), 423–433.

39 Hijaz, F.; Nehela, Y.; Gonzalez-Blanco, P.; Killiny, N. Development of Europium-Sensitized Fluorescence-Based Method for Sensitive Detection of Oxytetracycline in Citrus Tissues. *Antibiotics*. **2021**, *10*(2), 224.

40 Jamalaiah, B.; Kumar, J.; Babu, A. M.; Moorthy, L. R. Spectroscopic studies of Eu^{3+} ions in LBTAF glasses. *J. Alloys Compd.* **2009**, *478*(1–2), 63–67.