High fluorescent carbon dots/Ag as a sensitive sensor for tetracycline waste in aqueous solution

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Abstract. Currently, antibiotics waste produced by hospitals and pharmacies is increasing, increasing demand and public consumption. Tetracyclines are a popular type of antibiotic that can pollute the environment even in low concentrations. This study aimed to detect tetracyclines in an aqueous environment using C-dots/Ag. C-dots material was synthesized by microwave radiation method with the addition of Ag 0-4 %. C-dots/Ag is produced in the form of a brown-black powder that glows green. FTIR testing shows that C-dots/Ag contains various functional groups O-H, N-H, C-H, C=O, and C=C. The surface morphology of the C-dots based on SEM testing is round and rough. Ag's addition causes C-dots' surface morphology to agglomerate, shifting the C-dots' absorbance peaks towards a larger wavelength and decreasing the energy bandgap. C-dots/Ag 2% has the best optical properties based on the intensity of the resulting fluorescence. In general, C-dots/Ag has excellent potential to be a susceptible, selective, and effective tetracycline detection agent.

1. Introduction
Antibiotics are a type of drug that is believed to treat infections for decades [1]. The high consumption of antibiotics by the public can cause pollution and decrease environmental quality. Antibiotics can contaminate the environment when released from pharmacies and hospitals [2-4]. The antibiotics that are most commonly found on the market are sulfonamides (SA), quinolones (QN), macrolides (MAL), and tetracyclines (TC). Among these antibiotics, TC is the product most often found in water [5]. Part of the TC is excreted through feces and urine into the environment [6].

Fluorescent nanoparticles are currently being intensively developed as sensors because of their unique and attractive optical properties [7], simple, safe, and strong [8]. Carbon dots (C-dots) are a new class of nanomaterials that can emit fluorescence with low toxicity [9], so they can be applied as sensors for various pollutants [10], including TC [11-14]. The problem faced in using C-dots as a sensor is the low difference in luminous intensity produced when detecting TC. Therefore, additional material is needed to increase fluorescence so that the sensitivity of the C-dots better.
Efforts to increase C-dots’ sensitivity as a TC sensor can be made in combination with metal nanoparticles. So far, metal nanoparticles have robust sensing [15] and imaging [16] capabilities. Silver (Ag) as a metal nanoparticle has been proven to be used to detect heavy metal ions, anions, DNA, proteins, biomedical drugs, bacteria, and others [17], making it enjoyable to combine C-dots and Ag nanoparticles as sensors. C-dots can be synthesized by several methods, such as laser ablation [18], microwave irradiation [19], chemical carbonization [20], and hydrothermal [21]. Among these methods, the microwave irradiation method has become popular because of its lower cost than other methods. It can be achieved in one-step synthesis and narrow size-distributed through homogeneous heating [22-23].

2. Methods
The materials used are citric acid monohydrate powder (Merck KgaA), AgNO3 (Merck KgaA), urea (Merck KgaA), and aqua dest. The tools used are microwave reactor (Samsung MS28J5255UB), batch ultrasound (Krisbow 10039597), hotplate, magnetic stirrer, beaker, measuring cup, spatula, and mortar. The manufacture of C-dots/Ag begins with mixing 2 g of citric acid monohydrate powder and 3 g of urea powder in 60 ml aqua dest with a procedure stage according to previous research [23]. The result of mixing was added 0-4% wt of AgNO3.6H2O. The solution is homogenized for 15 min at 70°C, followed by the sonication process using an ultrasonic cleaner at 40°C for 20 min. After that, the solution was irradiated in a 1000 W microwave oven for 30 min.

The surface morphology of C-dots/Ag was tested using scanning electron microscopy (SEM JEOL JEM-1400). C-dots/Ag functional group's content was tested using a Fourier transform infrared spectrometer (FTIR PerkinElmer Spectrum IR 10.6.1). C-dots/Ag fluorescent's optical properties were tested using an optical multichannel analyzer (OMA 405 nm). C-dots’ absorbance and bandgap energy were obtained through ultraviolet-visible photo spectroscopy instrument (UV-Vis Shimadzu 1240SA) testing. Antibiotic detection test using C-dots/Ag was carried out based on differences in the fluorescence intensity. C-dots/Ag 10 ppm dissolved in water and several antibiotics, namely tetracycline, doxycycline, cefadroxil, and amoxicillin.

3. Result and discussion
C-dots/Ag has been successfully synthesized using microwave assistance. The sample is a brown-black solid powder—this powder color results from the polymerization and carbonation processes in the reactor during the synthesis. Based on SEM testing, C-dots have around and rough surface morphology. Ag's presence causes C-dots' surface morphology to form aggregates and agglomerate, as shown in Figure 1. Ag particles appear to blend with Cdots so that they cannot be distinguished. The Ag particles are encapsulated in the middle of the carbon and distribute on the carbon shell. The Ag nanoparticles' distribution in the matrix and the carbon shell's surface comes from the outer diffusion of the Ag nanoparticles through electrostatic forces [24]. Based on the intercept technique, the average surface grain size of the C-dots is 24 nm.

Figure 2 shows the C-dots/Ag transmittance band at wavenumbers 3900 cm⁻¹ to 400 cm⁻¹. This C-dots/Ag FTIR spectrum indicates the presence of several functional groups of chemical compounds. In the 3000-3500 cm⁻¹ wavenumber, some peaks identify O-H and N-H groups. This result confirms that the C-dots produced have good hydrophilic properties. C-dots' hydrophilicity and stability in the solution will increase when there are O-H and N-H bonds (presence of hydroxyl and amine groups) [25]. There are also other functional groups on the C-dots/Ag surface, such as the C-H bond in the 2844 cm⁻¹, C=O in the 1634-1704 cm⁻¹, and the C=C bond which is a group alkene at the wavenumber of 1419-1465 cm⁻¹ [26, 27]. This functional group's existence is consistent with the results of previously reported research on the addition of Ag to carbon [28, 29]. Both the C-dots and C-dots/Ag exhibited similar characteristic absorption bands. This phenomenon was not only because the C-dots were derived from citric acid but also revealed that the functional groups were still present on the surface after carbonization.
The optical properties of C-dots/Ag were analyzed using a UV-Vis spectrophotometer. Figure 3 shows the intensity of the C-dots absorbance spectrum, which decreases with the addition of Ag. The prominent absorbance peak of the C-dots was observed to be at a wavelength of 408 nm. The addition of Ag on the C-dots causes a shift in the absorbance peak towards larger wavelengths (redshifted) up to 417 nm. The shift of the absorbance peaks towards larger wavelengths in the visible light region makes C-dots/Ag composites have great potential in fluorescence imaging [26]. Agglomerated C-dots/Ag causes electrons that approach the particle's surface to become delocalized and split with other particles that trigger the Surface Plasmon Resonance (SPR) to move to lower energy. This displacement causes the absorption peak to shift at a larger wavelength [30]. Apart from affecting the absorbance peak, Ag's addition also affects the decreasing energy bandgap value of C-dots, as shown in Table 1.

The success of C-dots/Ag synthesis can be observed based on the appearance of fluorescence. Qualitatively, C-dots/Ag produces green fluorescence, as shown in Figure 4a. This green fluorescence is consistent with previous studies' results using the same raw material [23,25,31]. Quantitatively, the C-dots/Ag fluorescence is measured with an Optical Module Analyzer shown in table 2. A higher peak intensity indicates an increased amount of photon emission produced by electron-hole recombination. The peak wavelength obtained from the five samples is followed by the resulting fluorescence, namely
green, which has a wavelength between 537-544 nm. C-dots' fluorescence intensity was observed to decrease with Ag's addition but increased in the 2% Ag doping variation. This phenomenon is probably due to the plasmon band coupling condition almost identical to fluorophore emission [32]. Based on the consideration that C-dots/Ag 2% has the highest fluorescence intensity, so the sample was chosen as the sensor material to detect antibiotic waste in this study.

![Figure 3. UV-Vis C-dots/Ag spectrum](image)

**Table 1. C-dots/Ag energy bandgap**

| Samples       | The energy bandgap (eV) |
|---------------|-------------------------|
| C-dots        | 2.670                   |
| C-dots/Ag 1%  | 2.657                   |
| C-dots/Ag 2%  | 2.664                   |
| C-dots/Ag 3%  | 2.664                   |
| C-dots/Ag 4%  | 2.654                   |

**Table 2. C-dots/Ag fluorescence intensity**

| Samples       | Intensity (a.u.) |
|---------------|------------------|
| C-dots        | 9069.25          |
| C-dots/Ag 1%  | 9100.25          |
| C-dots/Ag 2%  | 9636.50          |
| C-dots/Ag 3%  | 8866.00          |
| C-dots/Ag 4%  | 8670.50          |

C-dots/Ag was successfully applied to detect several antibiotic solutions, as evidenced by PL testing. The fluorescence sensor mechanism occurs based on a decrease in the fluorescence intensity of an analyte's presence. The results showed that the fluorescence of C-dots/Ag was quenched with the addition of several antibiotic solutions, as shown in Figure 4. The fluorescence sensor's ability to respond to tetracyclines can be seen from the decrease in intensity (turn off) that occurs from the addition of C-
dots / Ag. When compared with other antibiotics, tetracyclines show a quieter effect with a decrease in the emission spectrum. This phenomenon is due to the IFE (inner filter effect), which occurs because the tetracycline absorption band overlaps with the C-dots/Ag fluorophore excitation band. The presence of Ag doping can be a strong absorber in IFE. Ag nanoparticles’ plasmon absorption surface is susceptible to size, shape, the distance between particles, composition, and their environment so that the absorption spectrum can be adjusted to overlap with the emission spectrum of fluorophore [33]. The C-dots/Ag surface is filled with several functional groups, including carboxyl groups in which there are carboxylate ions. TC to C-dots/Ag can form hydrogen bonds due to various functional groups (amides, hydroxyl) present in TC. The hydrogen bonding between C-dots/Ag and TC increases the probability of electron transfer between them, which helps in dynamic fluorescence quenching of C-dots/Ag [34].

**Figure 4.** Appearance of C-dots/Ag fluorescence in (a) water (b) tetracycline

**Figure 5.** The decrease in the intensity of C-dots/Ag fluorescent on various antibiotics

C-dots/Ag decreased significantly in intensity from 9684.00 to 793.00 a.u. From this drastic decrease, it can be seen that the sensitivity of C-dots/Ag in responding to the presence of tetracyclines is very high compared to other antibiotics. The steeper the graph of the decrease in intensity with the addition of antibiotics shows that the more sensitive the fluorescence sensor is in responding to antibiotic analytes.
These results confirm that C-dots/Ag selectively and sensitively can be a detection agent (sensor) for antibiotic contamination, especially tetracyclines.

4. Conclusion
Synthesis of C-dots/Ag by microwave irradiation method produces brown-black powder with a round and rough surface morphology and produces a green fluorescence. C-dots/Ag contains various functional groups, such as O-H, N-H, C-H, C=O, and C=C. Ag’s addiction causes a shift in the C-dots’ absorbance peak towards a larger wavelength and a decrease in the energy bandgap's value. C-dots/Ag 2% has the best fluorescence intensity. In general, C-dots/Ag has excellent potential to be a sensitive and effective tetracycline detection agent.

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