Potential of Graphene Oxide-Like Material Derived from Corn Cob Waste as Adsorbent Phase of Dispersive Solid Phase Extraction for Determination of Tetracycline Antibiotic Residue

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Potential of Graphene Oxide-Like Material Derived from Corn Cob Waste as Adsorbent Phase of Dispersive Solid Phase Extraction for Effect of Tetracycline Antibiotic Residue

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Abstract. The significant increase in the use of tetracycline antibiotics has become a new threat to human health. Antibiotics that are not absorbed will be excreted through feces and urine into the environment so that they can cause gene changes, hormonal disorders and cause antibiotic resistance. The low analyte concentration and complex matrix of antibiotic residues require appropriate sample preparation techniques. This study aims to investigate the potential of graphene oxide-like material derived from corncob for solid phase of dispersive solid phase extraction (DSPE) method as one of the sample preparation techniques. The production of graphene oxide was carried out using a single step method without an oxidizing agent. Characterization of graphene oxide was performed using FTIR instruments to determine functional groups, and SEM-EDX to observe surface morphology and elemental composition. The optimum condition of DSPE method was conducted including variations in mass, pH, concentration, and contact time. The characterization results indicate that the synthesized graphene oxide possesses various functional groups, including oxygen, carboxyl, epoxy, carbonyl, and hydroxyl groups. Additionally, the material exhibits wrinkled surfaces and the X-ray diffraction analysis shows that the graphene oxide range typical for amorphous materials. The results showed that the optimum conditions were using single step graphene oxide at a mass of 8 mg with a concentration of 2 ppm at pH 5 and within 50 minutes. Based on this research, it can be inferred that graphene oxide-like material from corn cob waste show the high potential as solid phase od DSPE method for effect tetracyclic antibiotic residues with an absorption of 97%.

Keywords: Tetracyclinen, antibiotics, DSPE, Corn cob, graphene oxide

INTRODUCTION

The emergence of a pandemic due to the COVID-19 virus has led to the misuse and significant increase in the use of antibiotics, thus posing new threats to human health in the future [1,2,3]. Antibiotics are drugs used to treat infectious diseases. Inappropriate use of antibiotics will lead to antibiotic resistance [4]. Antibiotic residue is one of the emerging pollutant that has become the focus of attention with the Covid-19 pandemic outbreak, because this outbreak has triggered the massive use of antibiotics to treat and prevent the Covid-19 virus attack. Antibiotics that are not absorbed will be excreted through feces and urine into the environment so that it can cause gene changes, hormone disorders and cause antibiotic resistance [1,2]. Therefore, monitoring of residues of pharmaceutical products in the environment is very necessary for early warning and controlling the dangers of emerging pollutant. One of the residues of pharmaceutical products in antibiotics is tetracycline antibiotics.

Tetracyclines are a group of antibiotics that are widely used in the environment to treat various diseases caused by bacterial infections[5]. The use of tetracycline antibiotics in the long term is a serious problem for the environment which is considered a contaminant that enters the ecosystem [6]. Therefore, it is necessary to control tetracycline antibiotic residue in aquatic environments with efficient and cost-effective technology.

Effect of residues of pharmaceutical ingredients has been developed using various sophisticated instruments such as GC, LC, CE and ELISA [8,9,10,11]. However, the effect of residues of pharmaceutical ingredients in the environment is complicated because they are in a complex matrix and have low analyte levels, even lower than the detection limit of the tool [12]. Therefore, it is urgently needed the sample preparation technique efficient so that the sample matrix does not affect the performance of the instrument. So far, the preparation technique used is liquid-liquid extraction using a large number of solvents, procedures and a long time. To overcome this problem, a Dispersive Solid Phase Extraction (dSPE) technique has been developed. The dSPE technique has advantages such as faster preparation time, easy to perform.

The dSPE technique is based on the principle of equilibrium of analyte adsorption in the solid phase so that the choice of solid phase is a crucial factor. Currently, solid phases based on nanostructured materials such as fullerenes, carbon nanotubes (CNTs) and graphene have been developed to replace the conventional solid phase. Of the three groups, graphene and its derivative, graphene oxide, have the advantage that they can be synthesized more easily [14,15,16,17]. So far, graphene and its derivatives have been synthesized from non-renewable materials, are relatively expensive and require high synthetic costs. This has prompted various efforts to synthesize graphene oxide from alternative sources that are renewable, abundant and inexpensive, such as agricultural waste such as rice paddy, soybean dregs, cottonseed, straw, peanut shells [15].

Lampung Province is the third largest corn producer with corn production of 2.83 million tons with a harvested area of 474.9 thousand hectares, maize production in Indonesia increases every year [18]. Increased production of corn will result in increased corncob waste that has not been utilized optimally so that it has the potential to increase environmental pollution. Along with its development, corncob is used as an ingredient for making activated charcoal [19]. Corn cobs that have been processed into charcoal through the combustion process are used for the manufacture of nanotechnology, especially in graphene oxide (GO) synthesis.

Based on the description above, this study reveal potential of graphene oxide-like material derived from corn cobs as a dSPE material for determining tetracycline groups which are widely used in Indonesia.

MATERIALS AND METHODS

Materials and Equipment

The equipment used in this study include fume hood (*V Fume Hood*) centrifugation (*Fisher Scientific 1827001027164)*, pH meter (*Water Taster EZ-9901*), Muffle Furnace Up to 1400 ºC, while the analysis and characterization in this study used *Microscopy-Energy Dispersive X-Ray* (SEM-EDX) (*Perkin Elmer* 99951), Spektrofotometer *Ultraviolet-Visible* (Uv-Vis) *(Agilent Cary 100)* and *Fourier transform Infrared Spectroscopy* (FTIR) (*Agilent Technologies* FTIR 630 *CARY*).

The materials used in this study were corncob obtained from local farmer, standard tetracycline, solution 0.1 M NaOH (*Merck*™), 0.1 M HCl (*Smart-Lab*), and 2M HNO3 (*Supelco Sigmaaldrich*) solution.

Graphene Oxide Synthesis

Graphene oxide prepared using the single step method used by (Debbarma et al., 2019)[20]. Corn cob waste was cut into small pieces, then washed with deionized water several times to remove dust and dirt, then dried in the sun for 2 to 3 days, and dried in an oven for 24 hours at 70 °C. The dried corn cob was ground into powder, then pyrolyzed at 350 °C for 30 minutes. The black powder obtained from the pyrolysis process was washed using 2M HNO_3 solution to remove by-products, then washed using warm deionized water to remove impurities, then filtered using filter paper, and the precipitate obtained was dried in an oven for 24 hours at 70 °C.

Optimization of DSPE Method

Effect of Optimum Mass Adsorbent

Graphene oxide was weighed as much as 6, 8, 10, and 12 mg put into a beaker glass, added 20 mL of a standard solution of 10 ppm tetracycline. The resulting mixture was stirred for 30 minutes, then graphene oxide was separated from the solution by centrifugation at 10,000 rpm for 15 minutes. The solution was filtered using filter paper, the resulting filtrate was analyzed using a UV-Vis spectrophotometer at its maximum wavelength.

Effect of Optimum pH

Weighed 8 mg of graphene oxide was put into a beaker glass, added 20 mL of a standard solution of 2.5 ppm tetracycline with a pH of 3; 5; 7; 9; 12 using 0.1 M HCl solution or 0.1 M NaOH solution. The resulting mixture was stirred for 30 minutes, then graphene oxide was separated from the solution by centrifugation at 10,000 rpm for 15 minutes. The solution was filtered using filter paper, the resulting filtrate was analyzed using a UV-Vis spectrophotometer at its maximum wavelength

Effect of Optimum Adsorbate Concentration

Weighed 8 mg of graphene oxide put into a beaker glass, added 20 mL of standard solution of tetracycline pH 5 at different concentrations, specifically 2; 5; 7.5; 10 ppm. The resulting mixture was stirred for 30 minutes, then graphene oxide was separated from the solution by centrifugation at 10,000 rpm for 15 minutes. The solution was filtered using filter paper, the resulting filtrate was analyzed using a UV-Vis spectrophotometer at its maximum wavelength.

Effect of Optimum Contact Time

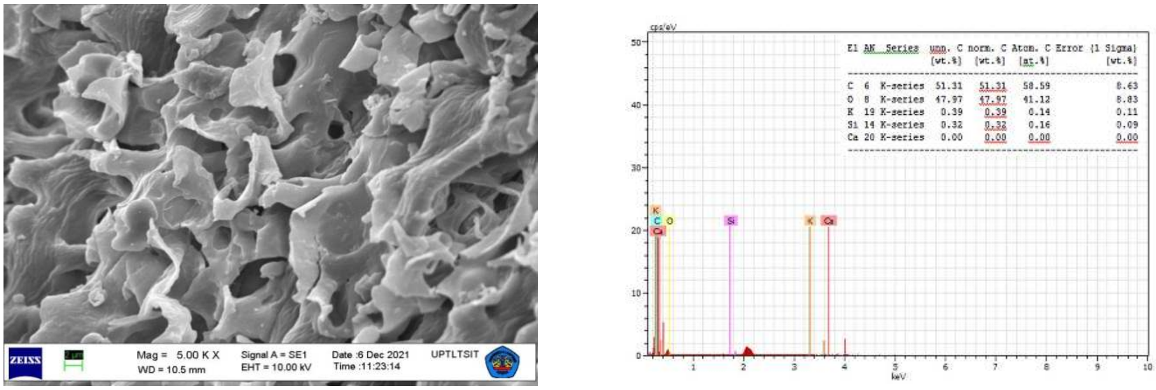
Weighed 8 mg of graphene oxide into a beaker glass, added 20 mL of standard solution of tetracycline pH 5 at a concentration of 2 ppm and with variations in time, namely 40, 50, 60 and 70 minutes. The resulting mixture of stirring was then separated graphene oxide from the solution by centrifugation at 10,000 rpm for 15 minutes. The solution was filtered using filter paper, the resulting filtrate was analyzed using a UV-Vis spectrophotometer at maximum wavelength.

RESULTS AND DISCUSION

Graphene Oxide Characterization

Characterization by Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM-EDX)

The characterization using SEM-EDX aims to determine the surface morphology and to determine the elements contained in the graphene oxide adsorbent. The results of the graphene oxide adsorbent analysis using SEM are shown in Figure 1.



(a) (b)

FIGURE 1. SEM test results (a) 5000x magnification (b) EDX spectrum on graphene oxide adsorbent.

Based on Figure 1 a. it is known that SEM micrographs reveal a rough-looking graphene oxide adsorbent surface and show homogeneously dispersed graphene sheets. The roughness or irregularity of an adsorbent surface can be interpreted as the presence of a porous nanosheet structure so that it will provide higher adsorption activity. Figure 1 b. Presents the percent by weight of the element (wt%) obtained from the EDX analysis. The synthesized adsorbents showed that the main elements were quite high, namely carbon (C) and oxygen (O) of 51.31 wt% and 47.97 wt% and very low elements of potassium (K) and silica (Ca), specifically 0.39 wt% and 0.32 wt%.

Characterization with a Fourier Transform Infrared (FTIR) Spectrophotometer

The absorption power of an adsorbent can be affected by the composition of the adsorbent in the form of functional groups. FTIR analysis is to identify the functional groups contained in the adsorbent so that it can be seen whether carbon has been successfully synthesized into graphene oxide. The results of graphene and graphene oxide adsorbent analysis using FTIR are shown in Figure 2.

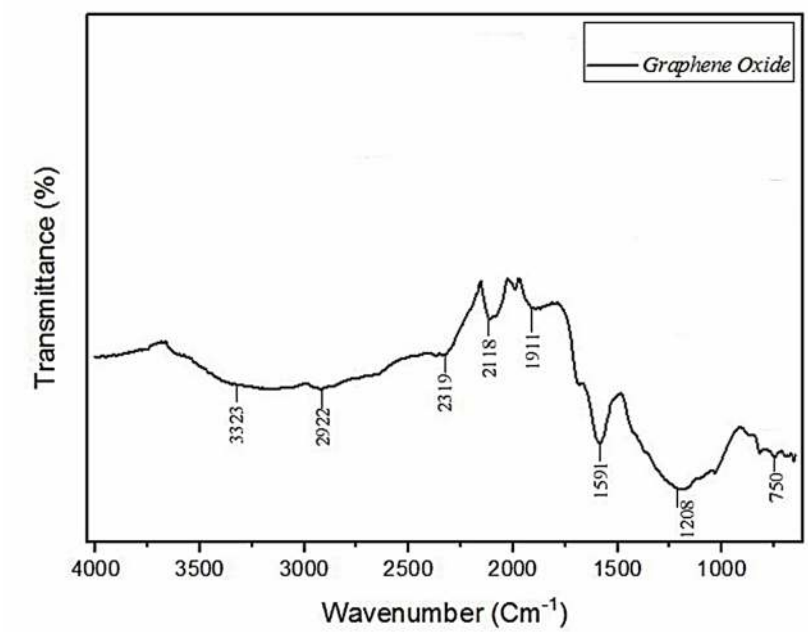


FIGURE 2. FTIR spectrum results (a) Graphene, (b) Graphene Oxide.

TABLE 1. match the functional groups of sample test results with references.

Datasheet[21]	Sample Graphene Oxide	Bond Type
900 - 670	750	*C-H Aromatic*
1310- 1020	1208	*C-O-C Stretching, ethers (aromatic, elefinic or aliphatic)*
1600 -1450	1591	*C=C Stretching aromatic ring*
2000-1280	1911	*C=O Stretching*
2500- 2000	2118;2319	*C≡C Stretching*
3000-2800	2922	*C-H stretching aliphatic*
3400-3200	3323	*O-H Stretch of hydrogen*

Graphene Oxide has the main functional groups namely C=C and OH functional groups and other functional groups owned by graphene oxide are hydroxyl groups (C-OH), epoxy (C-O-C), carboxylic acids (C(=O)OH), and carbonyl (C=O). The spectrum of wave numbers 3368. 3323 cm^{-1} indicates the presence of –OH groups. The -OH group that appears on the FTIR spectrum of this corncob indicates that the carbon still contains H2O. The C=C functional group is the basic structure of graphene oxide which binds to one another and forms a hexagonal where the double bond is a covalent bond [8]. Absorption at a wavelength of 1578.1592 cm^{-1} shows the presence of a C=C aromatic bond peak which is an indication of the formation of graphene oxide. The FTIR test results showed that the graphene oxide material made from corn cobs had been successfully synthesized.

Optimization of DSPE Method for Tetracycline Extraction

Effect of adsorbent mass

Effect of the optimum mass was carried out to determine the optimum mass of adsorbent that could be used to absorb tetracycline antibiotics. The adsorption test based on the effect of the mass of the adsorbent was carried out with variations of 6; 8; 10 and 12 mg. The results of the adsorption test which are influenced by the mass of the adsorbent can be seen in Figure 3.

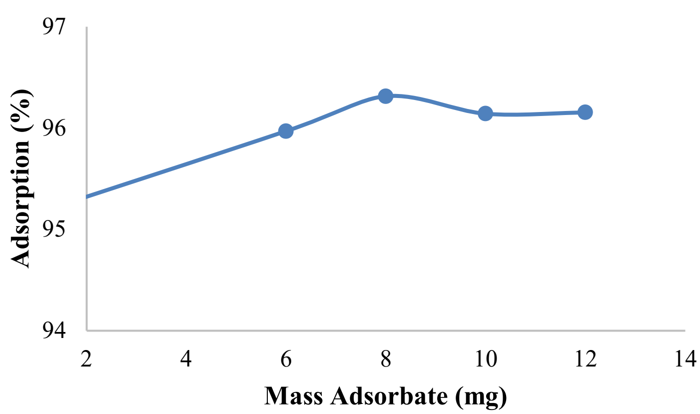


FIGURE 3. The results of the adsorption test are based on the effect of the mass of the adsorbent.

percen adsorption in this study can be used to calculate how much adsorbate in conventional tetracycline antibiotic solutions has been absorbed by graphene oxide adsorbents. Identification of the ideal state the mass of graphene oxide is 8 mg, and the adsorption rate is 96.25%. The use of adsorbent mass of more than 8 mg showed a decrease in adsorption causing an increase in density due to overlapping adsorbent particles, which prevented the active site of the adsorbent from being maximized during the adsorption process of the adsorbate resulting in a decrease in the absorption rate [22].

Effect of Optimum pH

Effect of the optimum pH was carried out to determine the optimum pH of the adsorbate that could be used to absorb tetracycline antibiotics. The adsorption test based on the effect of pH was carried out with variations of 3, 5, 7, 9, and 12 using the optimum mass of adsorbent that had been obtained. The results of the adsorption test which are influenced by the pH of the adsorbate can be seen in Figure 4.

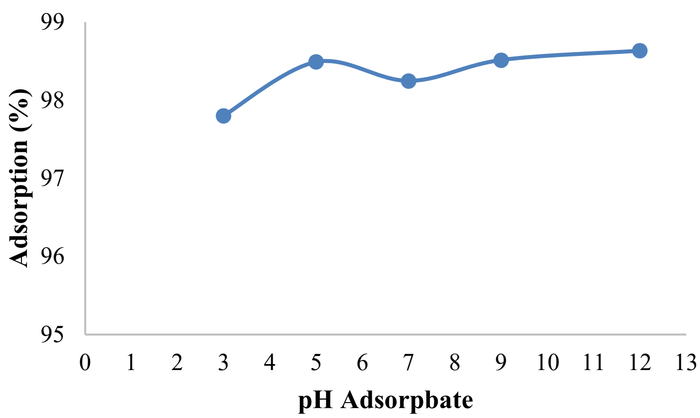


FIGURE 4.The results of the adsorption test based on the effect of the pH of the adsorbate.

The results of variations in the pH of the tetracycline solution are shown in Figure 4. The pH condition reached the optimum in this study, namely at pH 5 with an adsorption rate of 98.4%. The pH of the solution is an important parameter because it can change the charge on the graphene oxide surface and the types of ions in the solution. According to Abdulrahman (2020)[23] the optimum pH condition occurs between pH 2 to pH 10, because at this susceptible pH more functional groups are available and makes the electrostatic interaction between ions and graphene oxide stronger. Tetracyclines are almost cationic at low pH <4, zwitterionic in the pH range 5-7, and anionic at high pH. However, since all tetracycline species contain positively charged groups in their structure, it is possible that the molecules are arranged on the surface in such a way that the positively charged groups are very close to the surface, causing cationic bonds. Cationic bonds can occur between the easily protonated amino groups on the C ring of the tetracycline molecule and the electrons from graphene. Tetracycline adsorption on carbon nanotubes and graphite is due to the interaction of $\pi - \pi$ electron donor acceptors and cation-π bonds [24].

Effect of Optimum Concentration

Effect of the optimum concentration is carried out to determine the optimum adsorbate concentration that can be well absorbed by the adsorbent. The adsorption test based on the effect of the adsorbate concentration was carried out with variations of 2; 5; 7.5; and 10 ppm using the adsorbent mass and the optimum pH of the adsorbate that has been obtained. The adsorption test results which are influenced by the adsorbate concentration can be seen in Figure 5.

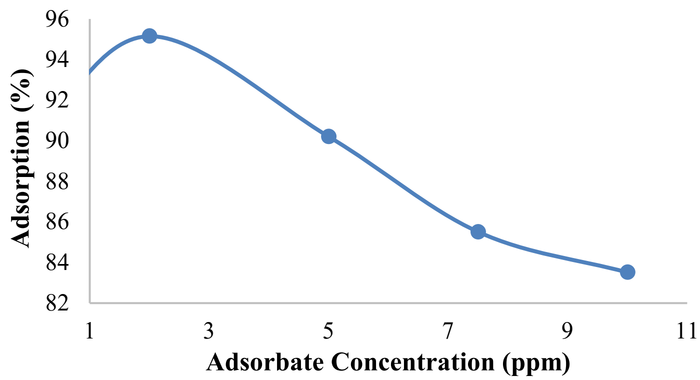


FIGURE 5. Adsorption test results based on the effect of adsorbate concentration.

The results of variations in the concentration of tetracycline solutions are shown in Figure 5. It shows that the adsorption process of tetracycline by graphene oxide has reached its optimum condition, namely at a concentration of 2 ppm with an adsorption rate of 95.14%. The decrease in the adsorption rate at concentrations of 5 ppm and above was caused by the adsorbent used having reached the saturation point so that the adsorption ability decreased and the contact time could cause the amount of adsorbate adsorbed to the surface of the adsorbent to increase and reach equilibrium. When equilibrium is reached, the surface of the adsorbent is filled with adsorbate so that the graphene oxide pores are covered by the adsorbate more increasing.

Effect of Optimum Contact Time

Effect of the optimum time was carried out to determine the optimum conditions for the interaction of tetracycline antibiotics in solution and their equilibrium state towards the adsorbent. The adsorption test based on determining the contact time was carried out with variations of 40, 50, 60, and 70 minutes using the adsorbent mass, adsorbate pH, and the optimum adsorbate concentration that had been obtained. The results of the adsorption test which are affected by contact time can be seen in Figure 6.

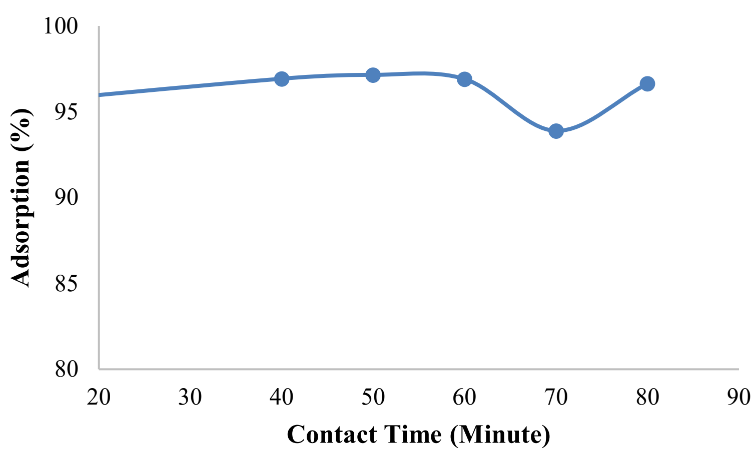


FIGURE 6. Adsorption test results based on the effect of contact time.

The results of variations in contact time in Figure 4 show that the adsorption process of tetracycline by graphene oxide reached optimum conditions at a contact time of 90 minutes with an adsorption rate of 97.15%. At a contact time of 60 minutes there was a decrease in the adsorption rate. The longer contact time between graphene oxide and tetracycline compounds causes the adsorption power of graphene oxide to be not optimal due to saturation in the adsorbent so that the active side of the adsorbate becomes less [25]

CONCLUSION

The results showed that graphene oxide from corn cobs has been successfully prepared and can be used as adsorption of tetracyclic antibiotic residues with an absorption capacity of 97%. Adsorption test of tetracycline antibiotics showed optimum conditions using single step graphene oxide at 8 mg adsorbent mass with 2 ppm adsorbate concentration at pH 5 with a contact time of 50 minutes, the formation of a porous nanosheet structure based on the results of SEM characterization, and having the elements carbon (C) and oxygen (O) which is quite high at 51.31 wt% and 47.97 wt% based on the results of the EDX characterization and the results of the synthesis of graphene oxide which were characterized using the FTIR test indicating the presence of OH and C=C functional groups which are the main functional groups that make up graphene oxide.

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