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# Design and Development of Graphene Oxide Modified by Chitosan Nanosheets Showing pH-Sensitive Surface as a Smart Drug Delivery System for Controlled Release of Doxorubicin

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***Abstract*****—**Drug delivery systems in which drugs are traditionally used, multi-stage and at specified intervals by patients, do not meet the needs of the world's up-to-date drug delivery. In today's world, we are dealing with a huge number of recombinant peptide and protean drugs and analogues of hormones in the body, most of which are made with genetic engineering techniques. Most of these drugs are used to treat critical diseases such as cancer. Due to the limitations of the traditional method, researchers sought to find ways to solve the problems of the traditional method to a large extent. Following these efforts, controlled drug release systems were introduced, which have many advantages. Using controlled release of the drug in the body, the concentration of the drug is kept at a certain level, and in a short time, it is done at a higher rate. Graphene is a natural material that is biodegradable, non-toxic, natural and wide surfaces of graphene plates makes it more effective to modify graphene than carbon nanotubes. Graphene oxide is often synthesized using concentrated oxidizers such as sulfuric acid, nitric acid, and potassium permanganate based on Hummer method. graphene oxide is very hydrophilic and easily dissolves in water and creates a stable solution. Graphene oxide (GO) has been modified by chitosan (CS) covalently, developed for control release of doxorubicin (DOX). In this study, GO is produced by the hummer method under acidic conditions. Then, it is chlorinated by oxalyl chloride to increase its reactivity against amine. After that, in the presence of CS, the amino reaction was performed to form amide transplantation, and the DOX was connected to the carrier surface by π-π interaction in buffer phosphate. GO, GO-CS, and GO-CS-DOX were characterized by FT-IR and TGA to recognize new functional groups which show the new bonding of CS to GO, RAMA and SEM to recognize size of layers that show changing in size and number of layers. The ability to load and release is determined by UV-Visible spectroscopy. The loading result showed a high capacity of DOX absorption (99%) and pH dependence identified as a result of DOX release from GO-CS nanosheet at pH 5.3 and 7.4, which show a fast release rate in acidic conditions.

***Keywords*****—**Graphene oxide, chitosan, nanosheet, controlled drug release**,** doxorubicin.

## I. Introduction

DRUG delivery systems (DDSs), in which drugs are consumed by patients in a traditional, multi-stage way and at specific intervals, do not fulfill drug delivery needs in the world. In today's world, we are dealing with numerous recombinant peptide and protein drugs, and hormone analogs in the body most of which are produced by genetic engineering techniques. Most of these drugs are used to treat vital important diseases such as cancer, diabetes, and autoimmune diseases. Traditional DDSs do not control the time, place, and speed of drug delivery. Furthermore, the drug concentration in the blood varies, may exceed the therapeutic range and cause less efficiency and more side effects [1], [2].

The drug concentration is kept at a certain level and the drug is delivered at a higher speed in a short time, using the controlled drug delivery in the body [3].

Graphene is a unique flat monolayer substance consisting of carbon atoms that are bonded in a two-dimensional and honeycomb-like lattice. The abnormal combination of its properties, such as very high hardness and mechanical strength, and high and adjustable electrical and thermal conductivity, has caused excellent optical and surface properties. Graphene is called a very thin monolayer graphite sheet that transmits light and has a transparency of 97.3% [4], [5]. Given the two-dimensional structure of graphene and the presence of free hands on this structure, this substance has a more active and effective surface compared to other carbon allotropes such as carbon nanotubes and fullerenes, which can carry out substitution and addition reactions and thus bonding with functional groups, and it can bond with functional groups from both surfaces and both ends; hence, it has more effective application in research works in which functionalization and its amount are important such as DDSs. Therefore, GO is very hydrophilic, easily dissolves in water, and creates a stable solution compared to graphene. Since the hydroxyl, carboxyl, and epoxy groups on the surface are very reactive, they can bond with functional groups and create new characteristics on the graphene surface [6], [7].

CS is synthesized from chitin which requires multiple chemical reagents. These polymers have outstanding properties such as non-toxicity, biodegradability, biocompatibility, eco-friendly, and safe, so CS is utilized in the pharmaceutical industry, tissue engineering, biomedical science, and engineering. The specific structure of CS allows absorbing the drug in solution sate [8].

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## II. Materials and Methods

### A. Materials

Graphene: It was prepared from Neutrino Company at a thickness of 4-20 nm, number of layers less than 30, purity greater than 99.5 (wt%), and diameter of 5-10 micrometers.

Oxalyl chloride, N,N-Dimethylformamide (DMF), phosphoric acid ($H_2PO_4$), sulfuric acid ($H_2SO_4$), potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), and hydrogen peroxide ($H_2O_2$) were prepared from Merck Company.

DOX was obtained from Sigma Company.

CS with a medium molecular weight was obtained from Sigma Company.

### B. Devices

- Heater Stirrer
- Centrifuge: made by Kokusan Company, Japan
- Oven: made by NÜVE Company, Germany
- Microwave: made by Milestone Company, Italy
- Scanning electron microscope (SEM): made by Hitachi Company
- Thermogravimetric Analyzer (TGA): made by Rheumatic company, model STE1500
- Fourier Transform Infrared Spectroscopy (FTIR): made by Thermo company, model Nicolet8700
- Raman Spectrophotometer: made by Thermo company, model Nicolet FT Raman 960
- UV-Visible device: made by Varian Company, model Cary100

### C. Method

1. Graphene Oxidation

First, 0.2 g of graphene is weighed and poured into a beaker, and then it is placed in an ice bath on a stirrer. 3 ml of phosphoric acid and 20 ml of sulfuric acid (98%) are added and allowed to stir at a temperature of below 0 °C for 30 minutes. Then, 0.25 g of sodium nitrate is added to the solution and allowed to stir at a temperature of below 0 °C for 30 minutes. 1.5 g of potassium permanganate is added slowly to the solution by controlling the temperature (the temperature must be below 0 °C) over one hour. It is stirred at 35 °C for 3 hours using a stirrer.

After conducting the above stages, 75 ml of distilled water is added to the solution under temperature control (it must be below 0 °C). Distilled water is poured until no purple vapor is seen.

The material is then placed in the ice bath on the stirrer, and 120 ml of a solution containing $H_2O_2$ and distilled water (20 ml of $H_2O_2$, 120 ml of distilled water) is gradually and slowly added to the solution. After completing this step, the product is placed in an ice bath and the temperature is controlled (it must be below 0 °C) and allowed to be stirred for 3 hours.

After this step, the resulting precipitate is allowed to settle and the remaining precipitate is washed with 50 ml of 5% hydrochloric acid and centrifuged. Then, the precipitate is washed with distilled water 6-7 times to neutralize the high solution pH of the precipitate.

After complete washing, the precipitate is poured into a container and placed in an oven at 60 °C to dry [9], [10].

2. Chlorination of GO

First, 3 three-neck round-bottom flasks are placed on the stirrer in an ice bath, and 0.08 g of GO and 25 ml of DMF are added to each of them and allowed to cool at a temperature of below 0 °C for 30 minutes. Then, the necks of the flasks are completely closed to make the flasks isolated, and 8 ml of Oxalyl chloride solution is slowly and gradually added to each of them through a Pasteur syringe by controlling the temperature (it must be below 0 °C). When the Oxalyl chloride is finished, the resulting solution is allowed to stir at a temperature of below 0 °C for 3 hours to complete the chlorination reaction. After completing this stage, the systems are placed on the heater at 70 °C, the lids of the flasks are opened so that the unreacted oxalyl chlorides leave the operating space, and this work continues until the solution becomes a completely dense liquid.

3. Amination

After the completion of the previous step on the flask prepared from the closed reflux system and CS, 0.4 g of CS is obtained and it is refluxed at 110 °C for 100 hours after closing the flask lid. After completing 100 hours of reflux, the flasks are placed in a 700-W microwave at 90 °C for 150 minutes to complete the reaction. Thereafter, the solution is poured into the test tube and washed with 98% ethanol, and the process continues until the supernatant becomes colorless. The precipitate is then poured into the crystallizer and put in the oven at 60 °C until it dries completely.

4. Drug Loading

0.02 g of the sample prepared at the amination stage is taken and poured into the Erlenmeyer flask and 2 mg of the drug (DOX) is added to it and 50 ml of phosphate buffer is then added to each. Erlenmeyer flasks are placed on the stirrer and stirred for 48 and 72 hours. After the end of every 24 hours, the substances are ultrasonicated for 1 hour, and the UV spectrum is obtained after centrifugation.

5. Drug Delivery

All the solutions are first centrifuged to completely separate the phosphate buffer from the precipitate for drug delivery. The precipitates are then completely dried, and 50 ml of phosphate buffer at pH 7.2 is poured on it. The resulting solution is stirred for 1 and 24 hours by the stirrer, and the UV spectrum is obtained from it at the end of each period. The solutions are completely centrifuged again and the remaining precipitate is dried. Then, 50 ml of phosphate buffer at pH 5.4 is poured on each and it is allowed to be stirred for 1 and 24 hours, and the UV spectrum is obtained after the end of each time interval.

## III. Results and Discussion

### A. IR Spectra of Graphene, GO, CS and GO Modified by CS

The observed signal at 3503 $cm^{-1}$ is related to the bending

vibration of absorbed water or the stretching vibration of OH on the surface. The signal at 1723 $cm^{-1}$ is also related to the presence of the carbonyl group on the surface of graphene. The signals at 1459.9 $cm^{-1}$ and 1633.69 $cm^{-1}$ are related to the C=C stretching vibration of the aromatic ring (Fig. 1).

The observed signal at 3420 $cm^{-1}$ is related to the presence of the OH group on the surface of GO. The signal at 1732 $cm^{-1}$ belongs to the presence of carbonyl groups on the surface of GO (Fig. 2).

The observed signal in the range of 3460 $cm^{-1}$ belongs to the stretching frequency of OH and NH of the CS ring. The observed signal in the range of 2869 $cm^{-1}$ is related to the C-H bond of the ring. The signal in the range of 1648 $cm^{-1}$ is related to N-H bond, and the signal in the range of 1097.6 $cm^{-1}$ belongs to the C-H bond (Fig. 3).

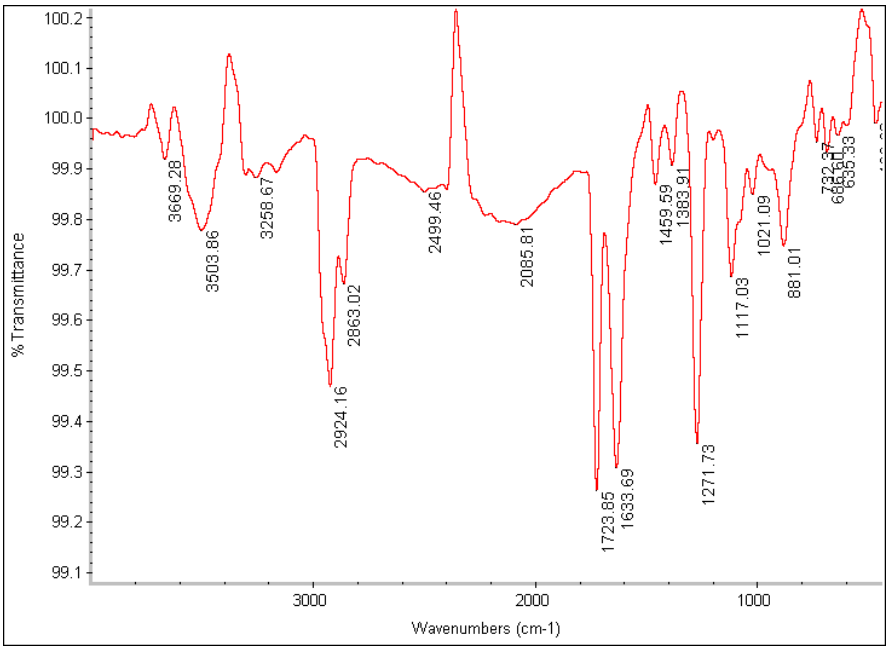


Fig. 1 IR spectrum of Graphene

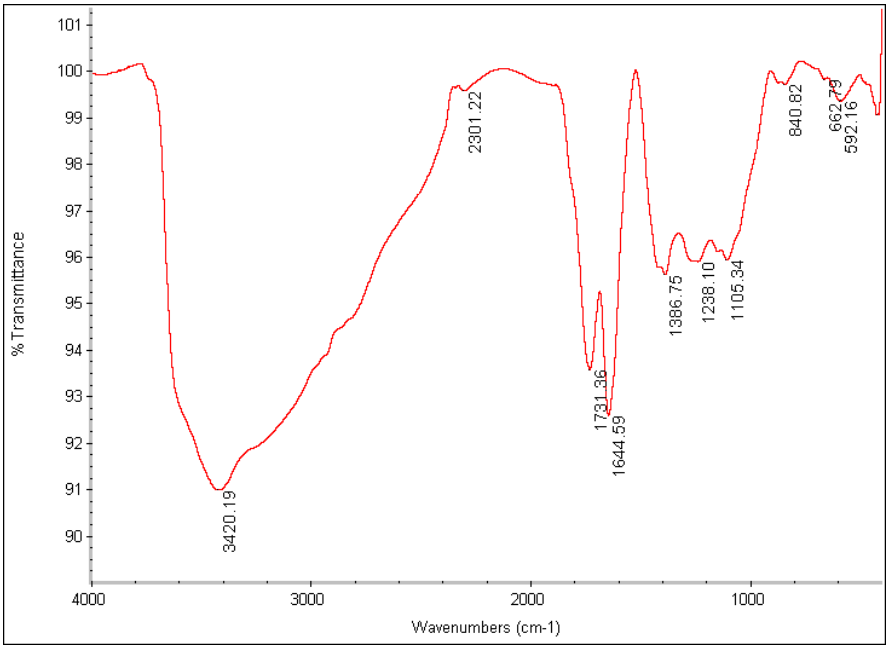


Fig. 2 IR spectrum of GO

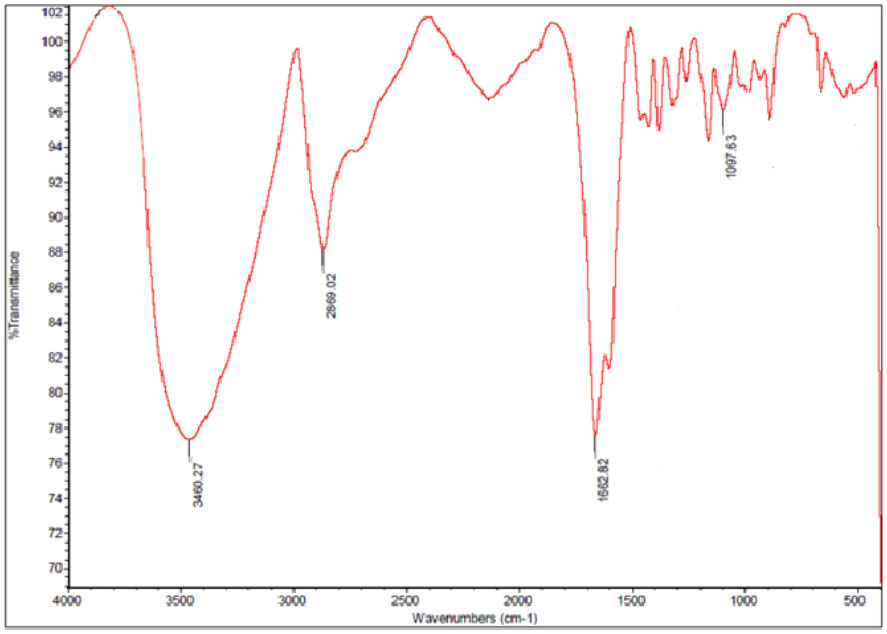


Fig. 3 IR spectrum of CS

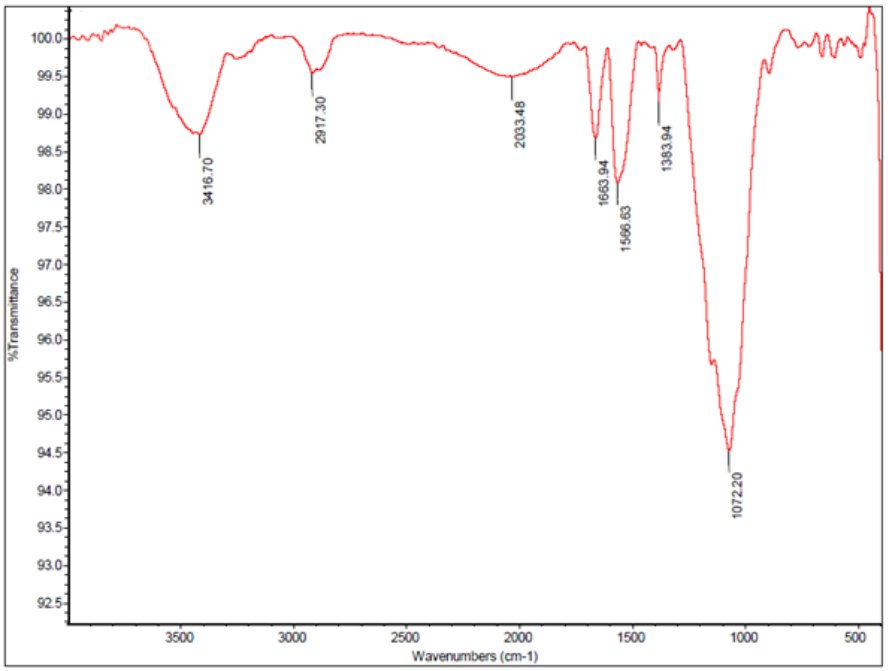


Fig. 4 IR spectrum of GO modified by CS

The observed signal in the range of 3416.70 $cm^{-1}$ is related to OH, NH groups of CS, and unreacted OHs of the GO surface. The signal in the range of 2917.30 $cm^{-1}$ is related to the C-H stretching vibration in the CS structure. The signal in the range of 1663-194 $cm^{-1}$ belongs to the carbonyl group bound to the N element in the structure of CS and the creation of amide which has changed its location compared to GO due to binding to the N element. The signal in the range of 1566.63 $cm^{-1}$ is related to the C=C bond of the aromatic ring of the GO sheet. The signal in the range of 1383.94 $cm^{-1}$ is related to the C-N bond and the signal in the range of 1072.120 $cm^{-1}$ belongs to the C-O-C bond or epoxy groups on the surface of GO (Fig. 4).

*B. Raman Spectra of Graphene, GO and GO Modified by CS*

The observed peak at 1319.06 $cm^{-1}$ is related to the D band and the observed peak at 1598.21 $cm^{-1}$ belongs to the G band, and the $I_D/I_G$ ratio is about 0.82, indicating that graphene is multi-layered (Fig. 5).

The observed peak in the range of 1292.02 $cm^{-1}$ is related to the D band and the peak in 1587.01 $cm^{-1}$ belongs to the G band, in which ID/IG ratio is about 0.94. An increase in the value of this ratio in comparison with graphene indicates the reaction

and also the monolayer of GO (Fig. 6).

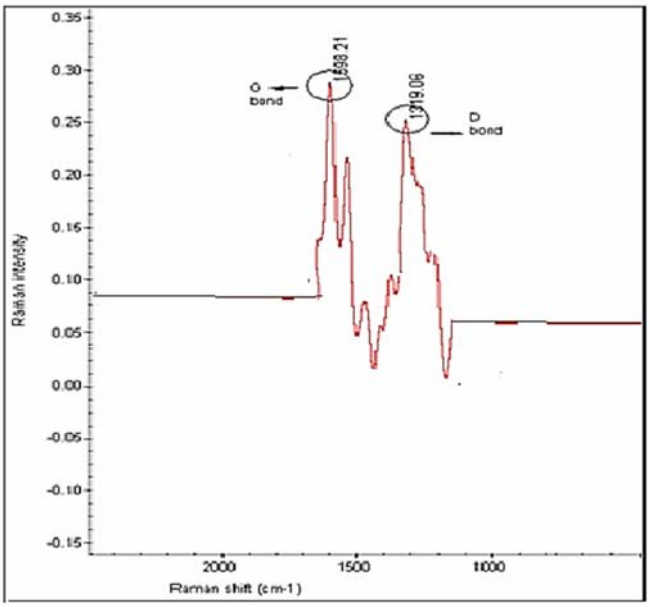


Fig. 5 Raman spectrum of Graphene

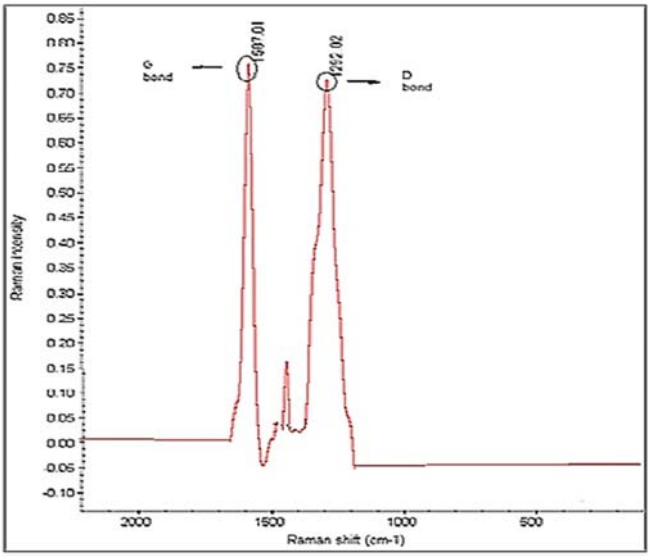


Fig. 6 Raman spectrum of GO

The observed peak in the range of 1260.17 $cm^{-1}$ is related to the G band and the peak observed in the range of 1544.94 $cm^{-1}$ belongs to the D band, in which the $I_D/I_G$ ratio is about 2.27 and an increase in this value compared to GO indicates that the reaction is done (Fig. 7).

*C. TGA Spectrum of GO Modified by CS*

A weight loss of about 13% at a temperature of 150 °C is related to the evaporation of the solvent, and a weight loss of about 32% at about 350 °C is related to the destruction of the oxidizing agents of GO. A weight loss of about 40% at about 580 °C belongs to the destruction of the CS ring. The remaining 5% is ash (Fig. 8).

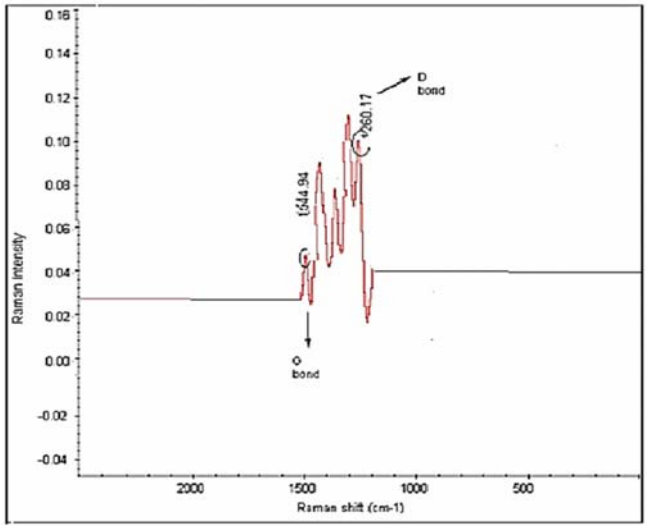


Fig. 7 Raman spectrum of GO modified by CS

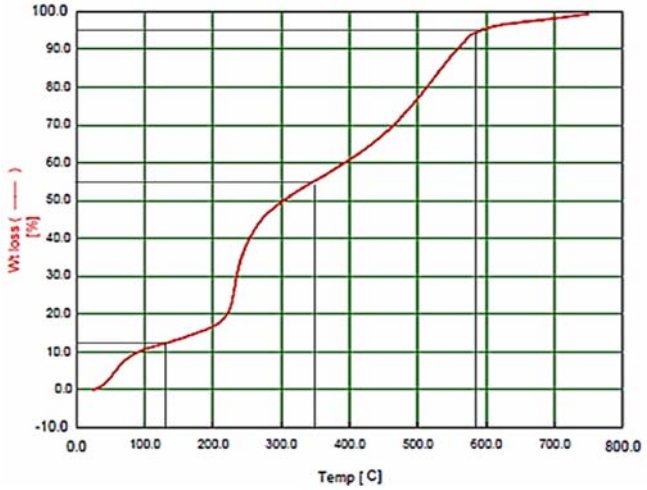


Fig. 8 TGA spectrum of GO modified by CS

*D.SEM Images of Graphene, GO and GO Modified by CS*

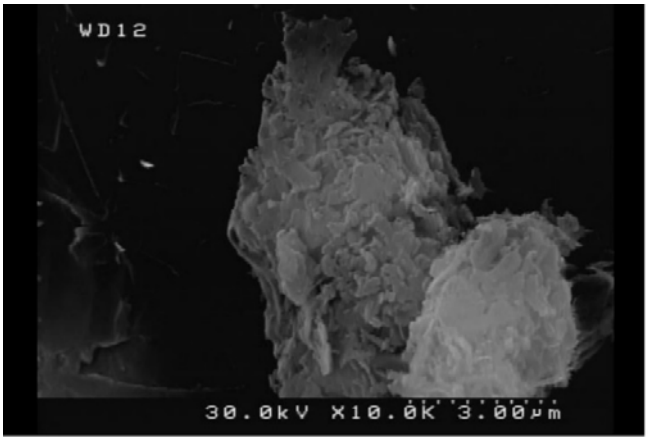


Fig. 9 SEM images of Graphene

In SEM mages of graphene, graphene sheets are visible on top of each other. The average thickness of the sheets is 18 nm in these images (Fig. 9).

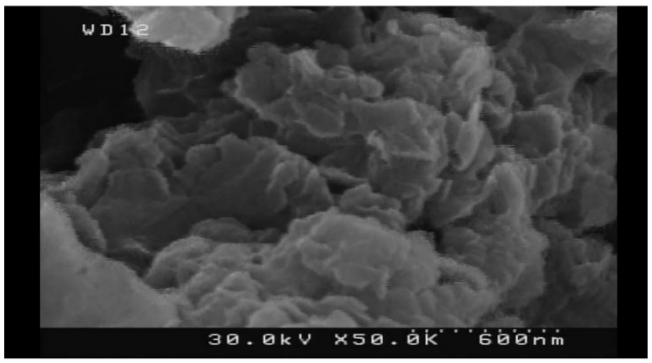


Fig. 10 SEM images of GO

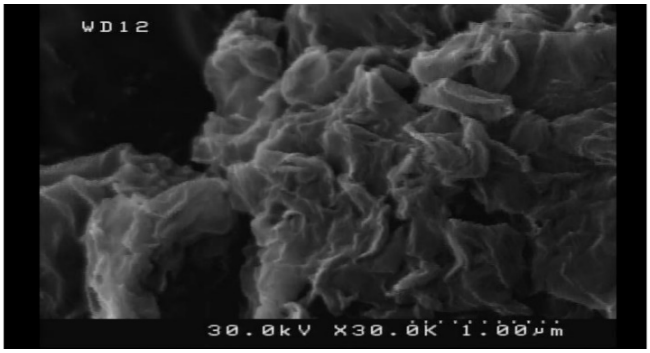


Fig. 11 SEM images of GO modified by CS

In SEM images of graphene oxide, GO sheets are visible and on top of each other. The average thickness of the sheets is 20 nm (Fig 10).

Several sheets of GO can be seen in SEM images of graphene oxide modified by chitosan. The average thickness of the sheets is about 30 nm (Fig. 11).

## IV. Comparison of the Results

### A. Comparison of the Results of IR Spectrum

The signal in the range of 1723 $cm^{-1}$ in GO is related to the carbonyl graphene group. This signal in the range of 1732 $cm^{-1}$ in GO appeared with greater intensity. This increase in intensity is due to increased oxidation. This value reached about 1663.194 cm in substituted GO, which was caused by the attachment of the carbonyl group to CS and the formation of amide. No changes were observed in the signal of OH and NH groups.

### B. Comparison of the Results of the Raman Spectrum

The ID/IG ratio in graphene was equal to 0.82. This value indicates that graphene is multi-layered. This value reached 0.94 in GO, which indicates the oxidation of graphene. This value reached 2.27 in substituted GO, which shows that the substitution reaction of GO was done.

### C. Comparing the Results of SEM Images

The SEM images of graphene showed that the thickness of the layers of graphene is about 18 nm and the sheets are stacked upon each other. In GO, the thickness of the layers increased to 20 nm, which indicates the deposition of functional groups due to oxidation on the surface of graphene, but the layers are still stacked upon each other. In the substituted GO, the thickness of the plates increased to about 30 nm. This increase shows the connection of CS with the surface of GO. In the substituted GO, the sheets are separated from each other and the transverse connections are increased compared to graphene and GO, and the sheets are seen as layers.

## V. Conclusion

In this research, GO substituted with CS was used as a drug carrier. First, Hamer's method was used to oxidize graphene and add oxygen-containing groups to increase its solubility in aqueous solvents. GO was then chlorinated by oxalyl chloride and three different concentrations of CS (0.3, 0.4, and 0.5 grams) were added to convert amine to amide. The DOX is loaded on GO substituted by π-π bond in a PBS environment (a drug with the concentration of 40 ppm and carrier with different concentrations of 100, 200, and 400 ppm were placed in a PBS environment for 48 and 72 hours). After the drug loading stage, to determine the release rate of the absorbed drug, the carriers attached to the drug are placed in a PBS environment with two different pHs for up to 48 hours.

The results obtained from the drug release stage show that the PBS environment is acidified due to the binding of hydroxide ions with the NH group of the drug surface so, the hydrogen bonds and intermolecular force are reduced, and as a result, the release of the drug from the absorbent surface is more in an acidic environment than in the neutral environment. On the other hand, as the time increases, the amount of drugs released in the environment increases, and after a while, absorption and release are done at the same rate. Therefore, over time, the release occurs very little. According to these results, it can be concluded that the prepared carrier is very suitable for drug release in an acidic environment.

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