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Drug Loading on Carbon Nanotubes synthesized by Flame Fragments Deposition Technique

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ABSTRACT
Doxorubicin (DOX) is widely used for anticancer, and it regards as one of the effective therapeutic drugs for solid tumors, such as carcinomas, sarcomas, and hematological malignancies. In this work, multiwall carbon nanotubes (MWCNTs) were selected as carriers to load DOX. The concentrations of (20mg) functionalization -CNTs as a nanocarrier were added to 100 ml of DOX aqueous solution (100 ppm). Each mixture was stirred for 24 hours at 25°C under dark conditions. The functionalization CNTs/DOX mixture was isolated by centrifuging for 15 min at a rate of 6000 rpm. Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM) were used to investigate the characterization of the nanocarrier. The results showed that the used dosage of CNT (1mg/ml) showed good loading ability for DOX over CNTs.

Keywords: Drug delivery; doxorubicin; multiwall carbon nanotubes (MWCNTs); flame fragments deposition; SEM; FTIR.

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INTRODUCTION

Doxorubicin (DOX) belongs to the anthracycline classification of chemotherapeutic agents that are of use in treating several common human cancers such as aggressive non-Hodgkin's lymphoma [1, 2]. Yet, the toxicity of DOX is rather high in humans, which eventually results in severe suppression of hematopoiesis, gastrointestinal toxicity [3], i) and cardiac toxicity [4].

The term carbon nanotubes (CNTs) is used to refer to ii) nanomaterials that have a tubular structure and consist of carbon atoms that are linked with only an sp2 hybridization. References to CNTs started to increase iii) about 27 years ago when Lijima presented the first clear research [5], which marked the beginning of the wide attention paid to CNTs. As a result, much literature was devoted to synthesis, purification, characterization, and studying physicochemical properties [6, 7], many of which eventually paved the way for several successful attempts of using CNTs practically in different scientific fields.

The CNT patch is regarded as an essential step forward in the development of a programmable, transdermal drug delivery system used in the treatment of a wide range of syndromes and can be adjusted to a patient's needs in such a way that will enhance both therapeutic administration and efficacy. Dr. Hinds and his colleagues created a novel skin patch device to deliver nicotine based on an active layer of aligned carbon nanotubes (CNT) approximately 1.5-7 nm in diameter crossing through a solid polymer film [8].

CNT's could be regarded as antitumor agents and, when in combined with conventional drugs, they could improve their chemotherapeutic effect noticeably using advanced drug delivery system. An aqueous solution of functionalized SWCNTs exposed to a radiofrequency field (RF) exhibits efficient heating, and this characteristic is worked out by Gannon et al. in destructing human cancer cells in a noninvasive and selective thermal manner, keeping minimal to no toxic effect on normal cells [9]. For the induction of thermal cell death in a noninvasive manner, a photothermal effect could be employed as it presents essential data on possible therapeutic targets for treating pancreatic cancer. There are three main characteristics found in this nanoscale drug delivery system (DDS):

Using functionalized SWCNTs as a biocompatible platform for delivering therapeutic drugs or diagnostics;

Conjugating prodrug modules of an anticancer agent activated into its cytotoxic form inside the tumor cells upon internalization and in situ drug release;

Attaching tumor-recognition modules to the nanotube surface [9,10].

In this work, a detailed methodology for functionalizing CNTs with different functional groups of value for a variety of different purposes by Fourier Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscope (SEM) .

MATERIALS AND METHODS

Synthesis of CNTs by Flame Fragments Deposition method

Iraqi liquefied petroleum gas was used as a source for carbon, whereas oxygen gas has been utilized to create a natural combustion atmosphere for liquefied petroleum gas. Nitrogen gas has been used as an inert gas, which helped to control the nature of the combustion of liquefied petroleum gas. Another of its uses is to cool the instrument after each experiment. The process synthesis nanotubes demand combustion to be incomplete for liquefied petroleum gas. After the gases enter the kiln, the combustion took place, forming a yellow flame. The nanotube deposition occurred afterward on the surface of each crucible. The time needed to perform the full process is 30 min. The inside temperature for the locally made instrument is 180° C [11, 12].

Purification procedure of CNTs:

As reported in previous work [9], hydrogen peroxide has been used in the purification of the synthesized CNTs. Briefly, synthesized CNTs (100mg) were dispersed in an ultrasonic water bath (50 ml of H_2O_2) for one hour. The mixture was left in the refrigerator at 4°C for 24h, after which the solution was allowed to reach room temperature to be heated marginally to 50°C until all hydrogen peroxide was removed completely. The sample was washed with deionized water and dried at 80 °C for 4h. Finally, the routes followed a similar way, using acetone for the dry sample. The suspension was centrifuged for about 15 minutes afterward. The detached CNTs were then calcined at 500 °C for 2 h [11, 13].

(OH-COOH) Functionalization of CNTs

The functionalization of CNTs is a remarkable act, which could be put into use for the introduction of some functional groups into the surface as well as the development of its surface properties. To achieve this, CNTs (100mg) were suspended in 75mL of hydrogen peroxide (30 weight %) in a 100 ml round bottom flask which is equipped with a condenser, after which the dispersion was heated to 80 ℃ at reflux for overnight [12] as is shown in Figure (1b).

Amino Functionalization of CNTs

Amino-functionalization of CNTs [14] was performed through several stages, starting with a mixture of about 0.1 g CNTs and a large excess of ethylenediamine (50 mL) in a three-necked flask fixed with a condenser, followed by stirring and heating the mixture at 105 °C for 10 h under the protection of argon. Then, the mixture was cooled down to room temperature. After being left for layering, the upper ethylenediamine was removed and the residual mixture was washed by plentiful acetone.

Finally, the amino-functionalized CNTs were gathered through centrifugation and sufficiently dried at 60 °C, as shown in Figure (1a).

Loading of doxorubicin on f-CNT

The loading of doxorubicin on f-CNT [15] was performed starting by preparing a DOX aqueous solution (1mg/ml), and dispersing 20 mg CNTs into the DOX solution. The mixture was stirred under dark conditions for 24 hr and vacuumed slowly at room temperature for 3 hr. The DOX-CNTs were collected through centrifuging at (10.000 rpm) and washing it with phosphate-buffered saline (PBS)(pH=7.4) solution to eliminate the dissociative DOX. The obtained DOX-CNTs was a vacuum – dried at room temperature and stored through sealing for next use. To evaluate the loading efficiency of doxorubicin, the supernatant was collected, and the doxorubicin concentration in the supernatant was analyzed using a UV-visible spectrophotometer at 488nm. The effective concentration was found to be 100ppm, as presented in Figure (1c) and (1d).

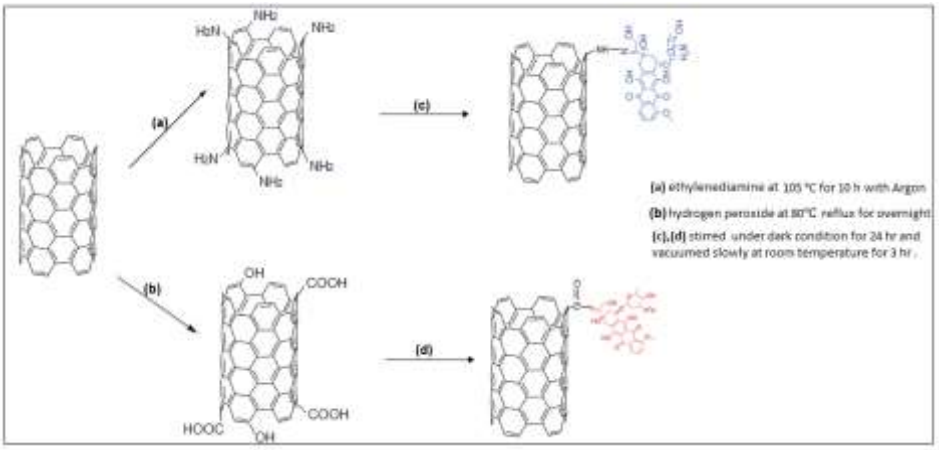

Figure 1: Functionalization of carbon nanotubes by two methods and loading DOX on CNTs

RESULTS AND DISCUSSION

Scanning Electron Microscopy (SEM)

Due to its three-dimensional representation, high resolution, and clear images, applying the scanning electron microscopy (SEM) technique could exhibit the dispersion of CNTs. Similar results were achieved by SEM analysis. Figure (2a) shows the images of the synthesized CNTs.

The morphological alterations in the sensing interface after treated amino-CNTs modification is shown in Figure (2b). This image reveals a homogeneous mesh of the amino-CNTs in the form of small bundles of tubes [16]. The adsorbed amino- CNTs resulted in a relatively higher roughness when compared to CNTs, by preferring an increased active surface over further antibody immobilization. SEM analysis also presented the chemical modification of the CNTs in the amino functionalization procedure, which effected the adsorption and formation of nanostructures.

The SEM images for CNTs-COOH and CNTs-DOX are displayed in Figures (2c) and (2d), respectively. The images show a clear morphology of these compounds. The image of CNTs-COOH reveals a smooth surface with curled and entangled tubes (Figure 2c), whereas the surfaces of carbon nanotubes in CNTs-DOX were rough with the existence of some attached clusters (Figure 2d). The phenomenon indicates that DOX macromolecular has been grafted on

the surface of CNTs through forming ester linkages between the reactive amino groups of DOX [17].

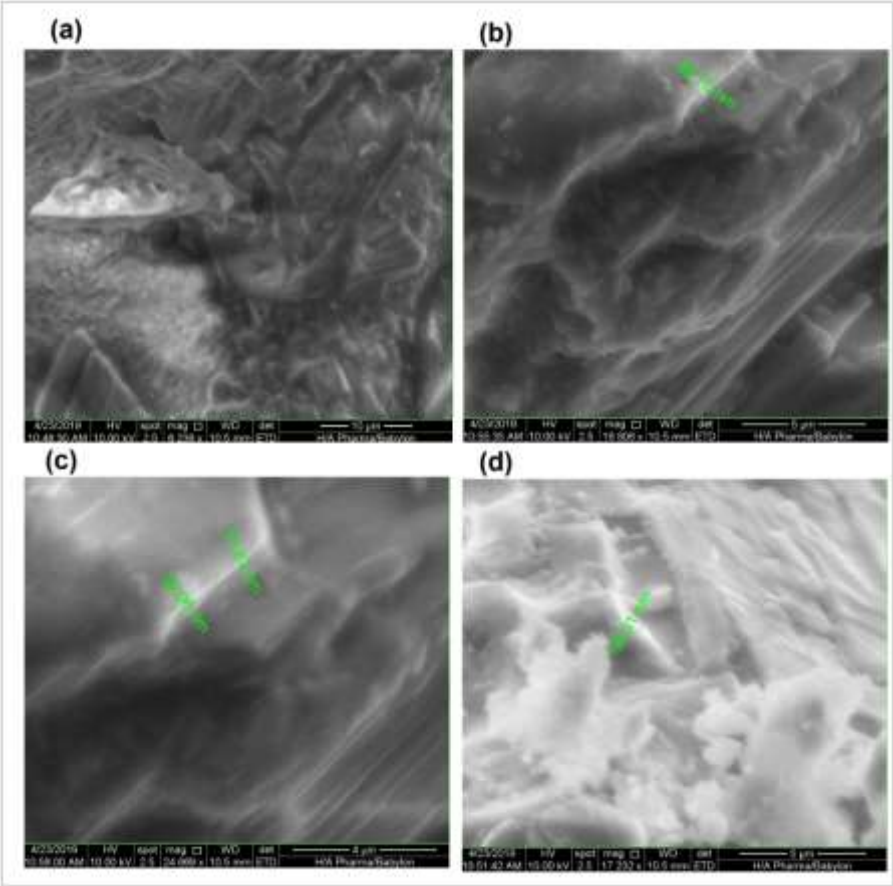

Figure 2: SEM of (a) synthesis CNTs, (b) Amino Functionalization of CNTs (c) (OH-COOH) Functionalization of CNTs, and (d) Loading of DOX on f-CNT

Fourier Transform Infrared (FTIR) Spectroscopy

One of the techniques used to achieve an infrared spectrum of absorption or emission of a solid, liquid, or gas is the Fourier-transform infrared (FTIR) spectroscopy. FTIR spectroscopy for acetone with an oil layer is shown in Fig. (3a) and (3b), and confirms the new chemical bands [11]. The influence of acetone with an oil layer produced by synthesized CNTs by (FFD) is shown in Fig (3c).

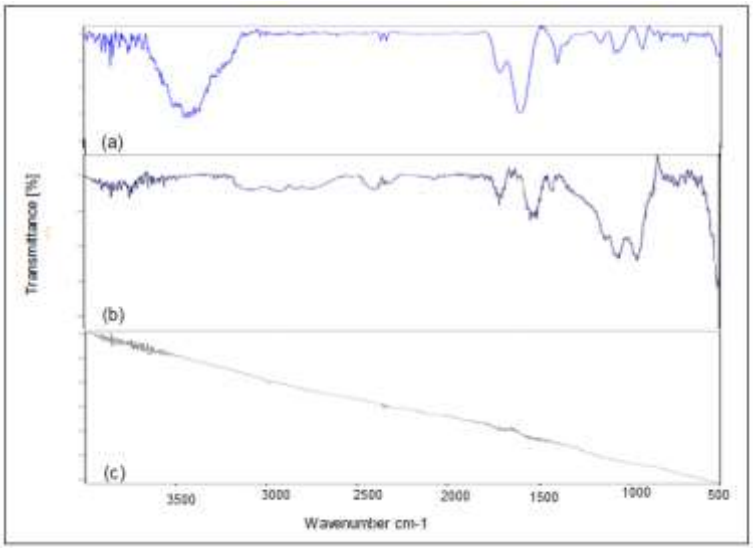

Figure 3: FTIR for synthesized CNTs (a) before purification, (b) after Purification by H_2O_2, and(c) after purification by (H_2O_2), aceton, calcined at 500 ^oC for 2 h

Table 1: Comparison of the FTIR for synthesized CNTs after Purification and before purification

CNTs before Purification (wavenumber cm^{-1})	CNTs after Purification by H_2O_2 (wavenumber cm^{-1})
3455.9	2966.28
1716.67	1715.72
1608.23	1541.33
1398.65	------
1073.27	--------
933.87	963.32

FTIR spectroscopy tends to be an influential tool to realize the purpose of comprehensive characterization. Fig. (4a) shows an FTIR for functionalized synthesized CNTs by an amino group. In contrast with synthesized CNTs, all treated samples show several peaks according to functional groups that are attached to their surfaces. The peaks shown at the ranges of 1554-1700cm^{-1} and about 3400cm^{-1} are consistent to –N-H bending and stretching vibration of amine groups. As well, the peaks at the range of 1060-1070cm^{-1} belong to the C-N stretching vibrations. The peaks in the regions 3420 and 3170 cm^{-1} can be attributed to the N–H stretch of the amino group. Besides, two peaks found at around 2995cm^{-1} and 2989 cm^{-1} prove the C–H stretching mode of the ethylenediamine molecule [18]. The appearance of new absorption bands at 1520 and 1340 cm^{-1} corresponds to the N–H stretch of the amino group. The peak at 1120 cm^{-1} is attributed to the C–N stretching of amide groups [19,20]. The presence and location of the –NH_2 and C–N bands in this spectrum presents significant evidence of the introduction of ethylenediamine moieties onto the CNTs. Then, the obtained results showed a successful method for amino functionalization of the CNTs. The chemical oxidation of CNTs is performed using H_2O_2 with UV in order to obtain the hydrophilic surface structure of oxygen within a certain surface group. This oxidation process with H_2O_2 introduces several functional groups including -OH (Hydroxyl), -COOH (carboxyl), and others, on the CNTs' surface. These results were used to control the oxidation -functionalization procedure [21]. Figure (4b) shows typical bands of the carboxylic groups at 3445 cm^{-1} corresponding to the molecular stretching of –OH groups. The presence of the small peak at 1710 cm^{-1} ascribed to the C=O stretching.

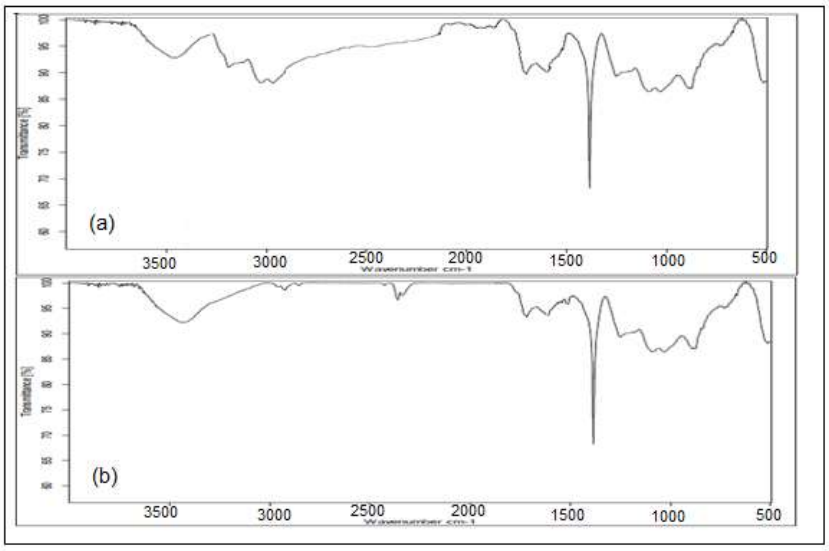

Figure 4: FTIR for (a) functionalized synthesized CNTs (-NH), and (b) functionalized synthesized CNTs (-OH, COOH)

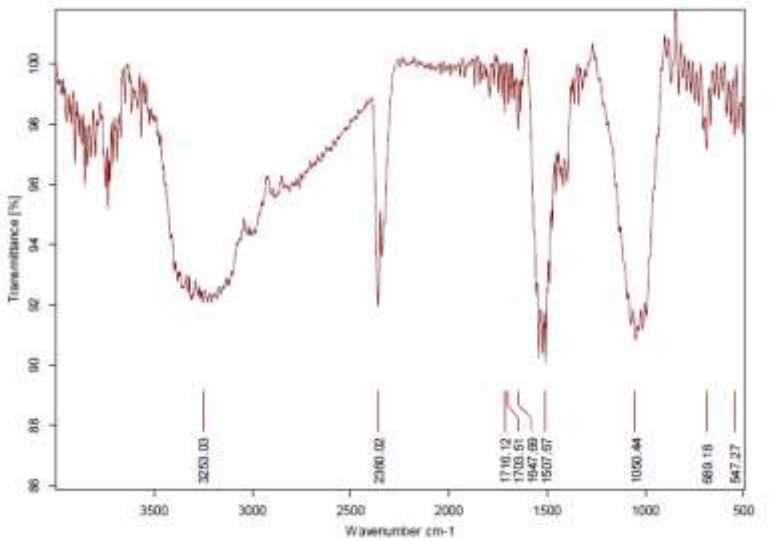

Figure 5: FTIR for Doxorubicin drug

The FTIR spectra of doxorubicin is shown in Fig. (5). The bands obtained at 3430 cm^{-1} to OH and peaks at 2926, 1715, 1674, 1507, and 1050 cm^{-1} are assigned to quinone and ketone carbonyl groups. The peak at 2926 cm^{-1} is because of the stretching bands of the C–H groups, The peak at 1716 cm^{-1} a result of the stretching bands of the C=O groups, the peak at 1610 cm^{-1} is attributed to the bending bands of the N–H groups, The peaks at 1051 cm^{-1} and 984 cm^{-1} are due to the stretching bands of the C=O and the C–O–C groups respectively. Finally, the peaks at 872 and 760 cm^{-1} are due to the primary amine NH_2 wag and N–H deformation bonds [22].

Figure (6) compares the FTIR for (a) functionalized synthesized CNTs (-OH, -COOH), FTIR for Doxorubicin drug, and FTIR for loading Doxorubicin drug on O- CNTs.

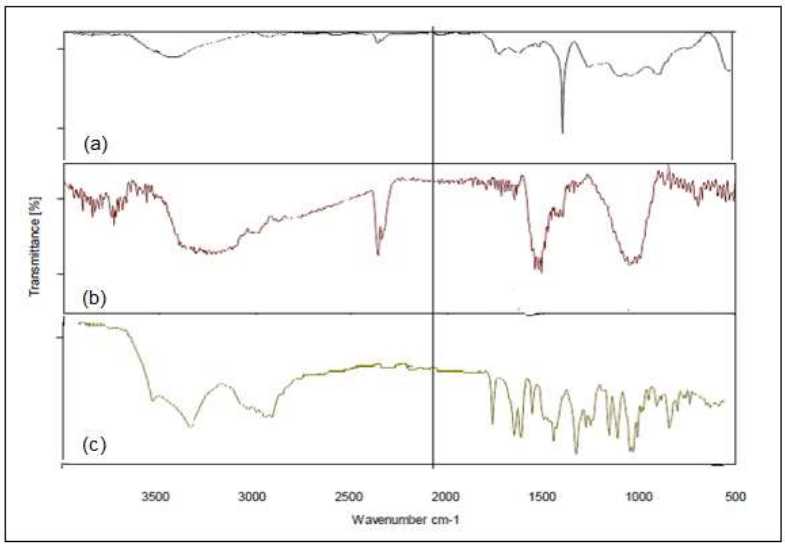

Figure 6: FTIR (a) functionalized synthesized CNTs (-OH, -COOH), FTIR for Doxorubicin drug, and FTIR for loading Doxorubicin drug on O- CNTs.

Figure 7 compares the FTIR for (a) functionalized synthesized CNTs (-NH), FTIR for Doxorubicin drug, and FTIR for loading Doxorubicin drug on N- CNTs.

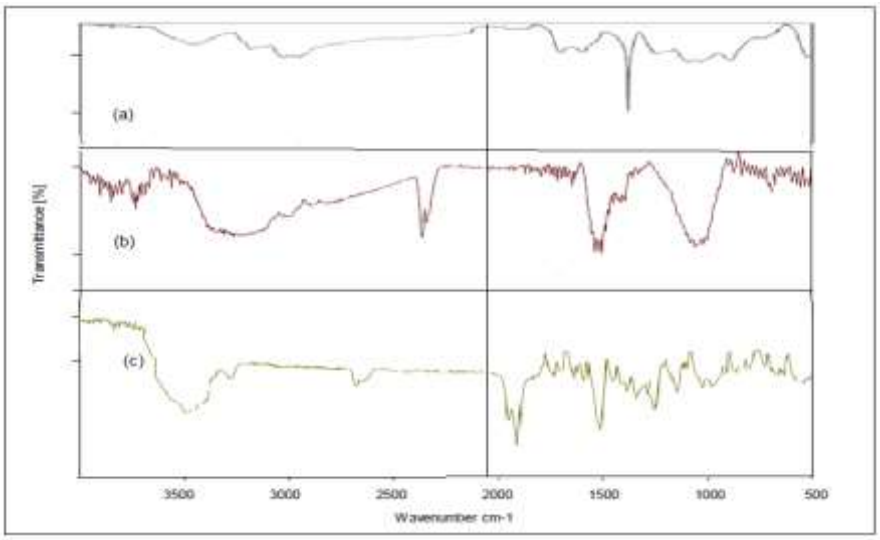

Figure 7: FTIR (a) functionalized synthesized CNTs (-NH), FTIR for Doxorubicin drug, and FTIR for loading Doxorubicin drug on N- CNTs.

Drug-loading results

The determining of the Dox-loading efficiency of samples [23] was as described below:

Dox-loading capacity = Weight of DOX /Weight sample DOX on f-CNTs

Table 2: Doxorubicin (Dox)-loading capacity of OH, COOH-CNTs, and NH_2-CNTs carbon nanotubes with a fixed weight of 0.0184 mg

Sample	Weight of Dox	Weight of sample	Drug-loading capacity
NH_2	0.00952mg	0.0184mg	0.517
OH-COOH	0.00891mg	0.0184mg	0.484

Table 2 shows that NH_2-CNTs had the highest drug-loading efficiency. Due to hydrophobic interactions between the NH_2 group and the drug, NH_2-CNTs had a remarkably higher efficiency in encapsulating drugs than COOH or OH CNTs. In addition, extra space on the NH_2-CNTs permitted drugs to attach to the MWCNTs walls through hydrophobic interactions and π–π stacking. OH, COOH-CNTs had the lowest drug-loading efficiency, due to the bulkiness of OH, COOH-CNTs are bulkier, which might have inhibited the attachment of DOX, as is shown in Fig. (13).

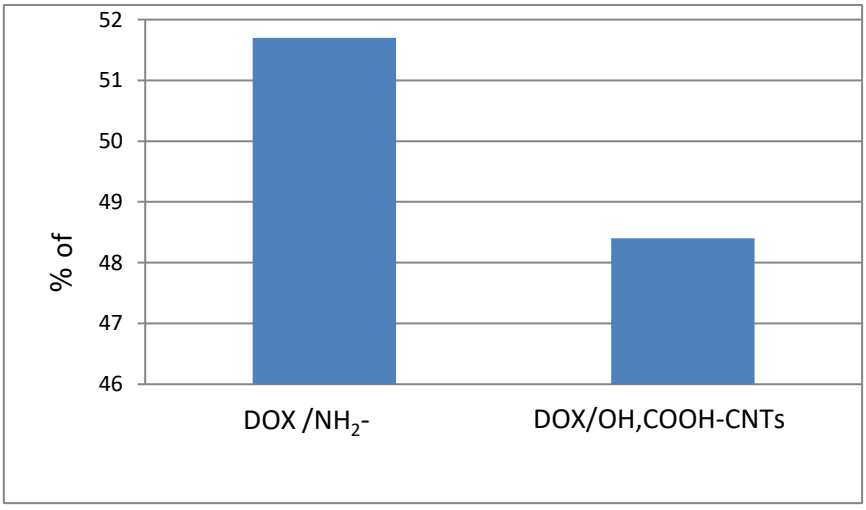

Figure 8: Drug-loading efficiency on f-CNTs

CONCLUSIONS

From the obtained results, it can be concluded that all used doses of CNTs provided good loading ability for DOX on CNTs. Yet, loading doxorubicin on CNT was performed by starting with preparing a DOX aqueous solution (1mg/ml) and dispersing 20 mg CNTs into the DOX solution.

Highly functionalized NH_2-CNTs and less functionalized CNTs (O-CNTs) were tested in comparison with pristine CNTs. The drug Doxorubicin -loading efficiency NH_2-CNTs (51.8%) increased than O-CNTs (48.4%)

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