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Original Article

ADSORPTION OF ERLOTINIB TO MULTIWALLED CARBON NANOTUBES

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ABSTRACT

Objective: The objective of this research was to assess for the first time the adsorption of erlotinib (ERL) in three types of multi walled carbon nanotubes, as feasible alternative method to removal antineoplastic from wastewater.

Methods: Both multi walled carbon nanotubes without modification (pristine-CNT) and modified carbon nanotubes by oxidation (CNT-COOH) and amination (CNT-NH_2) were used as adsorbents. They were characterized by Transmission electron microscopy, Raman spectroscopy, FT-IR spectroscopy, and Thermogravimetric analysis. In addition, the stability of CNTs suspensions were monitored. The ERL residual concentration in the equilibrium, from the bath adsorption, experiments was quantified by HPLC. The experiment data were fitted to Langmuir and Freundlich models.

Results: The characterization showed that the surface of pristine-CNT was modified. Different sedimentation behavior was observed in the three types of CNTs. ERL adsorption followed the Langmuir model for CNT-NH_2, Freundlich model for CNT-COOH and did not fit any models for pristine-CNT. Adsorption parameters were favoured with the functionalization of CNTs, which can be explained by properties of ERL and surface chemistry of CNTs.

Conclusion: It was found that CNTs have a high capacity of adsorption of ERL, indicating the potential of CNTs to removal this antineoplastic drug from hospital wastewater.

Keywords: Erlotinib, CNT, Adsorption isotherm, Antineoplastic

INTRODUCTION

Since some pharmaceuticals have been found in surface water at a concentration around ng/l-µg/l in different areas around the world [1], a concern arises about the fate of human drugs in the environment. The antineoplastic are probably one the drug with more eco-toxicological risks because they have potent action cytotoxicity, genotoxicity, mutagenicity and teratogenicity, and they are able to damage any eukaryotic cell [2]. Efforts as the program Cyto Threat (EU, 2011–2013) have been conducted to generate knowledge about the impact of the anticancer agent in the environment.

Some anticancer drugs act by a universal mechanism and, therefore, representing a major ecological risk due to the receptor is expressed in eco-organisms [3]. Protein kinases inhibitors, as Erlotinib (ERL), belongs to this sort of antineoplastic, its action is owing to block the proliferative signalling pathways through epidermal growth factor receptor (EGFR). ERL is the main therapy in cell lung cancer and pancreatic adenocarcinoma [4]. To date, there is scarce information on the effect of ERL on the ecosystem.

The world-wide prevalence of cancer rises and leading to increasing in the use of antineoplastic and, therefore, the ecosystem effects will come to be ever more important. The risk also will increase because it is becoming more frequent the outpatient consumption of the anticancer drug, as the case of ERL, that is orally bioavailable. This indicates that antineoplastic agents enter to the aquatic environment not only from hospital effluents but also from domestic effluents. A chlorination process normally carried out in wastewater treatment plants was proposed as a method to the removal of ERL. Nevertheless it was found chlorinated transformation products that could exhibit a negative environment impact [5].

With the awareness of the detrimental impact of drug traces in eco-species, it is clear the need to implement alternative treatment systems to wastewater before release them into the public sewage. The adsorption technology has great potential in this field, being one of the most powerful absorbents the carbon nanotubes (CNT) due to extremely high loading capacity as result of theirs ultrahigh surface area, up to 2600 m^2/g [6], their hollow monolithic structure, and rich surface chemistry which enables to simultaneously attach a wide range of molecules. In fact, they are one of the nanoparticles with a larger capacity of drug entrapment [7].

CNTs are an allotropic form of carbon; their structure consists of a layer of graphite wrapped around itself CNTs have interesting properties mainly due to the structural conformation that gives them great versatility and potential for use in different applications [8], despite the short time that has elapsed since their characterization by the Japanese scientist Iijima in 1991 [9].

The objective of the present study was to assess for the first time the capacity of CNTs to adsorb ERL from the aqueous medium. ERL was choice as a representative type of new antineoplastic agents. Three sorts of CNTs were evaluated, with different surface chemistry and, therefore, different interaction with ERL was expected. The findings of this study might to be critical to establish the feasibility of using CNTs in the removal of the anticancer drug from wastewater.

MATERIALS AND METHODS

Materials

Multi-walled CNTs were purchased from Chengdu Organic Chemicals Co. Ltd., (China), with a purity>95 wt%, outer diameter 8 nm and length 10-30 µm, SSA 500m^2/g. ERL was obtained from Chemical Land 21(South Korea). Methanol grade analytical and HPLC, potassium phosphate, ethylene diamine,1-ethyl-3-(3-dimethylaminopropyl)-Carboiimide (EDAC), cysteamine, concentrated sulfuric acid, concentrated nitric acid and sodium hydroxide were purchased from Merck and used without further purification. Ultra filtration membranes of regenerated cellulose with a cut of 10K Dalton (Millipore). Ultrapure water (18.2 MΩ cm) was obtained from a Synergy® Millipore system.

Methods

Functionalized-CNT preparation

Three types of CNT were tested, namely: pristine-CNT, carboxylated CNTs (CNT-COOH) and aminated CNTs (CNT-NH_2).

The oxidation was performed using oxidizing acids [10]. The amination was carried out by cross-linking methods in aqueous medium (environment-friendly) previously described, using the COOH groups as attachment points [11].

Finally, the CNTs were dispersed with water and ultra filtered, after which the filtered solid residue was washed extensively with excess water. The functionalized CNTs (CNT-COOH or CNT-NH_2) were dried in an oven at 60 °C for 24 h.

Characterization methods

TEM micrographs were made up by transmission electron microscope using a JEM1400Plus (JEOL, Japan) microscope at 60 kV. Samples were placed on carbon-coated grids; magnification was 100.000X-200.000X.

The FT-IR spectra were acquired using an IR Tracer-100 SHIMADZU spectrometer (Shimadzu Corp., Japan). The spectra were collected from pressed KBr pellets loaded with CNT and under ambient atmosphere uncontrolled. The samples were scanned from 400 to 4000 cm^{-1}.

Raman spectra were recorded on JASCO NRS-3100 Laser Raman Spectrometer (JASCO Inc., Japan) with an excitation radiation at 514 nm.

Thermogravimetric analyses (TGA) were performed on Instruments Hi-Res TGA(TA Instruments Inc. USA). The sample was heated at a 10 °C/min ramp, from 0 up to 900 °C under oxidized atmosphere.

Zeta potentials were measured using a Zeta sizer Nano ZS instrument (Malvern Instruments Ltd., UK), the samples of CNTs were suspended in unionized water.

An acid–base titration technique using sodium bicarbonate 0.05 M was performed to determine the percentage of COOH groups on the surface of oxidized CNTs. A sample of 50 mg of CNTs was added into a 50 ml 0.05 N $NaHCO_3$ solutions. The mixture was shaking using ultrasonic bath during a 1 hour. The CNTs were removed by filtration through a 0.1 µm nylon membrane. The excess $NaHCO_3$ in the solution was titrated with a 0.05 N HCl solution, monitoring the pH with a pH-metro ino Lab WT [12].

Batch adsorption experiments

All the adsorption experiments were conducted keeping the ratios ERL: CNT, 0.5:1, 1:1, 1.5:1, 2:1 and 3:1, which were calculated based on an equivalent of 10 mg of pristine-CNT.

Increasing amounts of ERL from 5 to 30 mg were added to 25 mL volumetric flasks, and solubilized with 10 mL of methanol due to ERL has a limited solubility in water (~0.4 mg/ml).

The f-CNT amount equivalent to 10 mg of pristine-CNT was added to each volumetric flasks, and the volume was completed with ultrapure water. These mixtures were dispersed in an ultrasonic bath for 45 min. Then they were placed on a magnetic shaker for 24 h at 30 °C at 300 rpm; subsequently the mixtures were kept at rest for 48 h at 30 °C in the dark.

The samples were centrifuged (4000 rpm, 30 min) using a centrifuge (Hettich Universal 320R) for separation of CNTs from solution. In order to quantify the residual ERL, an aliquot (3 ml) of the filtrate was placed in 25 ml volumetric flasks and diluted with methanol: phosphate buffer pH 3.5, 0.02 M, 1:1. Afterwards, it was analyzed by HPLC.

ERL analysis

The ERL residual concentration was quantified by HPLC on a SHIMATZU UFLC LC20 chromatograph (Shimadzu Corp., Japan), equipped with a LiChroCART® 250-4 RP-18 (5 µm, 250 mm) column. The mobile phase was methanol: phosphate buffer (pH3.5, 0.02 M), 35:65, the flow was 1 ml/min and the time analysis was 15 min, the wavelength of detection was 245 nm. The calibration curve was performed at 7 levels ranging from 0.01 to 0.16 mg/ml.

Data analysis

The adsorption isotherms were performed by determining the ERL residual concentration (Ce) and the qe (mg/g) capacity was calculated as:

$$qe = \frac{(Co - Ce)V}{m}$$

Where Co (mg/L) was initial concentration of ERL in the liquid phase, Ce (mg/L) was the ERL concentration in solution in the equilibrium, V is the volume of solution (0.025L), and m is the mass of CNTs (g).

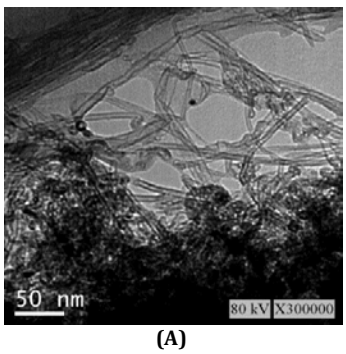
K_L and K_a, were calculated from the slope and intercept of the linear plot of C_e/q_e vs C_e. And K_f and 1/n were obtained from the intercept and slope of the plot of experimental data of lgq_e vs log C_e. The LM statistical package of R software was used.

Results and discussion

Multiwall CNTs were employed in this study because they are less expensive and more used in wastewater treatment than single-wall CNT, as well because multiwall CNT are less prone to aggregation than single-wall CNT [13].

Characterization of CNTs

Oxidation process led to modification of CNT surface and also to reduced CNT particle size as shown in TEM images (fig. 1).



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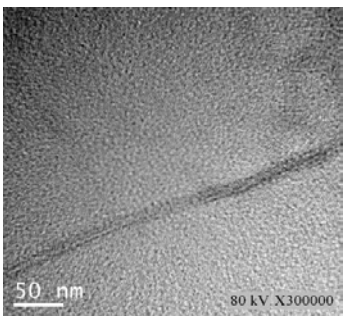


(B)

Fig. 1: TEM images of the CNTs: (A) pristine-CNT (B) CNT-COOH

Raman spectroscopy evidenced the increase in the number of defects in the walls of the CNTs during the oxidation process, by the D band-G´ bands intensity relation; which changed after oxidation because the intensity of D band enhances in regard to G´ bands (see fig. 2). The D-band (around 1330 cm^{-1}) is associated to either sp^3 carbon atoms or amorphous carbon, while G´-band (1590 cm^{-1}) is connected to sp^2 carbon atoms [14].

The formation of oxygen-containing functional groups such as carboxyl group during oxidation is evidenced by the signal at 3432 cm^{-1} in the IR spectrum, which is characteristic of the hydroxyl stretching from the carboxyl group and the signal at 1637 cm^{-1} assigned to the C=O stretch of the carboxylic acid group. The signals at 2933 and 2860 cm^{-1} have been assigned to stretching vibrations of CH_2, the adsorption peaks of C=C were observed at 1563 cm^{-1} and the

signal at 1107 cm^{-1} can be attributed to the (C–O) stretching vibration (fig. 3).

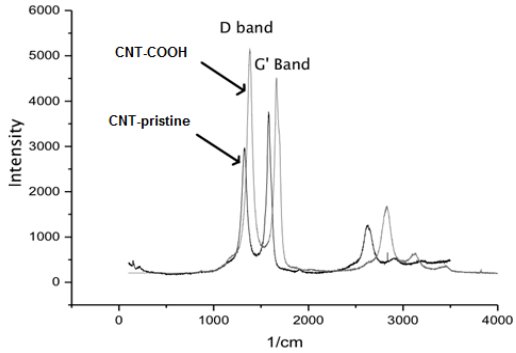


Fig. 2: D and G´ band in raman spectra

Respecting amino-functionalized CNT, the ethylenediamine covalent linkage to carboxyl group is evidenced by IR spectrum (fig. 3c) by the presence of a new bands at: 875 cm^{-1} which is representative of amino groups (NH_2), signals 1544 and 1049 cm^{-1} were attributed to N-H in-plane and C-N bond stretching, respectively, the weak peak at 3432 cm^{-1} has been assigned to the N-H stretching vibrations [15].

The thermal degradation of CNTs exhibits three stages in the thermogravimetric experiments,: a) range<150 °C, this weight loss is attributed to evaporation of absorbed water, b)150-350 °C, this stage corresponding to decarboxylation of COOH groups from the wall of CNTc)>500 °C is the weight loss due to thermal oxidation of the carbon.

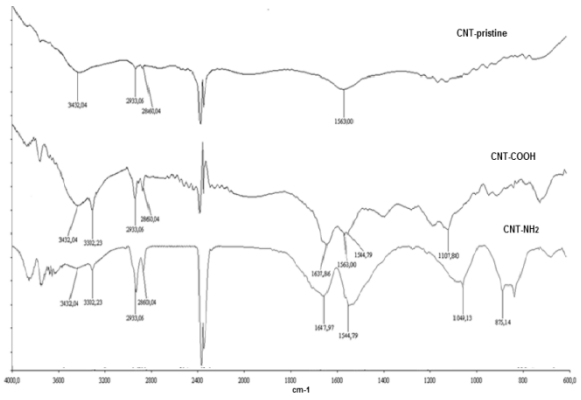
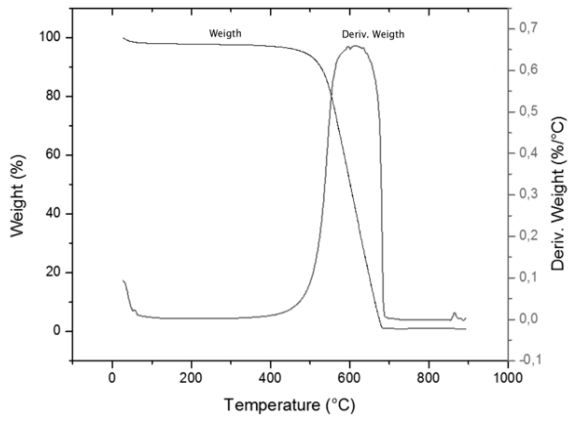


Fig. 3: IR spectra



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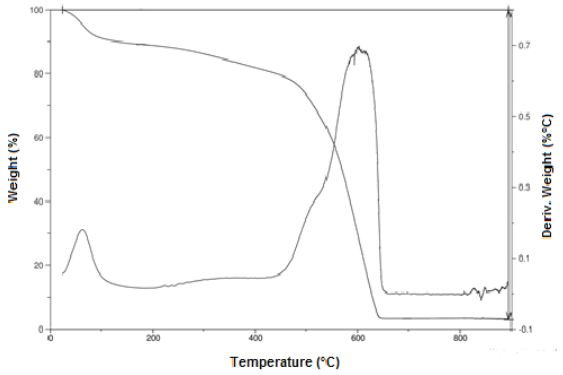

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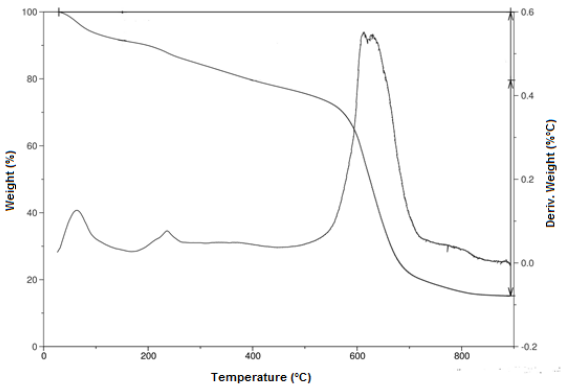


C

Fig. 4: Thermograms of A) pristine-CNT, B) CNT-COOH and C) CNT-NH_2

The thermogram of pristine-CNTs (fig. 4a) did not display the second stage, while the thermogram of oxidized CNTs (fig. 4b) exhibited a weight loss from 150 °C to 350 °C as was expected. The mg COOH groups per gram of CNTs was 78.8±3.20 according to titration assays of oxidized CNT. Aminated-CNTs (fig. 4c) showed a profile where the second stage was extended to 600 °C, which could be attributed to the decomposition of the ethylenediamine moiety [15].

Adsorption isotherm

The adsorption isotherms are important to design adsorption processes. At the present study the models of adsorption Langmuir and Freundlich were applied, under linear form:

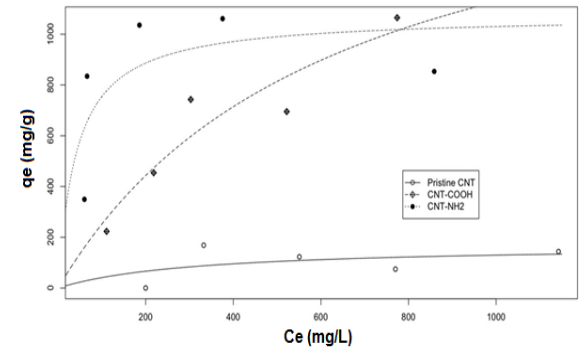
$$\frac{C_e}{q_e} = \frac{1}{K_L} + \frac{K_a}{K_L} C_e, K_L = q_m K_a \text{ ..... (2)}$$

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \text{..... (3)}$$

Where q_e (mg/g) is the ERL amount absorbed per unit weight of CNT in the equilibrium, Ce (mg/L) is the ERL concentration in the equilibrium in the liquid, K_L (L/g) is a Langmuir isotherm constant, q_m (mg/g) is the maximum adsorption capacity and K_a(L/mg) is a constant related to the free energy of adsorption in the Langmuir model;, K_f ($mg^{1-n}L^n/g$) is a constant indicative of the relative adsorption capacity of adsorbate according Freeundlich model, and n is the Freundlich exponential coefficient related to adsorption intensity of the adsorbent.

The experimental data are presented in fig. 5a in the plot of qe vs Ce. Figures 5b-c and 6 present the comparative fits to Langmuir and Freundlich adsorption isotherm of ERL on different kinds of CNTs. Table 1 summarizes the parameters found. Based on the higher value of R^2 (variation coefficient), experimental data from amino-functionalized CNTs were best fitted with Langmuir isotherm, which is corroborated by the similarity between the values of parameters q_e expand q_m. In the case of oxidized CNT, the data were reasonably

described by the Freundlich, isotherm model. Concerning for pristine-CNT both models failed.



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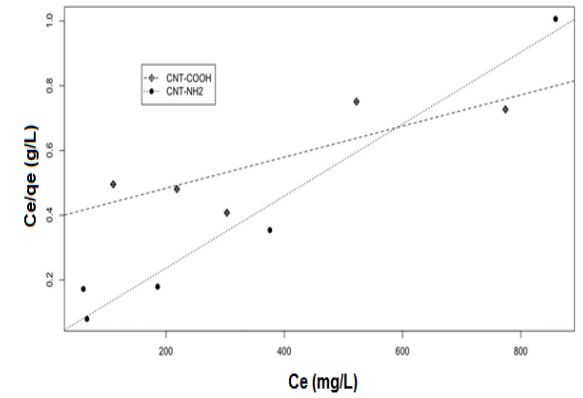

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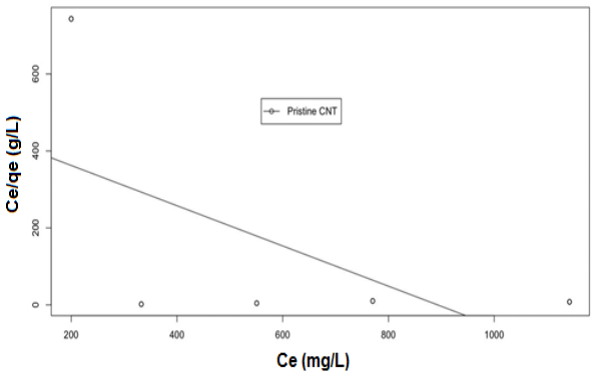

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Fig. 5: Langmuir adsorption isotherm for ERL on different CNTs at 300K.○pristine-CNT, ● CNT-NH_2, ♦ CNT-COOH

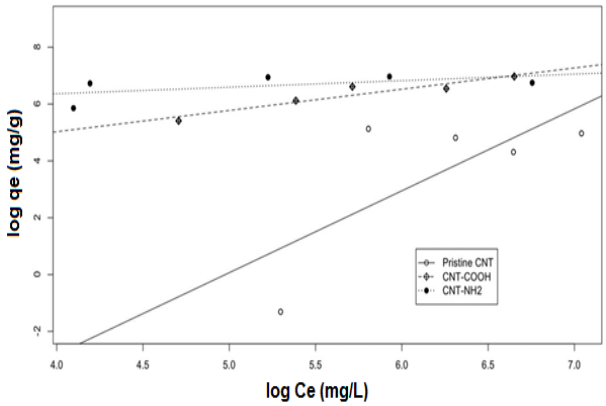


Fig. 6: Freundlich adsorption isotherm for ERL on different CNTs at 300K.○pristine-CNT, ● CNT-NH_2, ♦CNT-COOH

Also, the equilibrium parameter (R_L) was calculated, which is derived from Langmuir model according to equation 4

$$R_L = \frac{1}{1 + KLCo} \ldots\ldots\ldots\ldots (4)$$

R_L indicates whether the adsorption is irreversible (R_L=0), favorable (0<R_L<1), linear (R_L=1) or unfavorable (R_L>1). The values found were 0.73, 0.38 and 0.02 for pristine-CNT, CNT-COOH, and CNT-NH_2 respectively, suggesting that the adsorption was favorable [16].

The parameters of Langmuir and Freundlich were higher in CNT-COOH and CNT-NH_2 than pristine-CNT, suggesting the adsorption affinity and capacity were favored on the functionalized surface of CNTs. For example, it was observed that n>0 in all cases, and considering ranges given by Xiao *et al.*, 2014 [17] for this Freundlich parameter (n<1 poor, 1<n<2 moderate, 2<n<10 good adsorption), the absorption of ERL on CNT turned moderate to good with the functionalization. This trend has also been observed with hydroxyl and amino-substituted aromatics compounds, where non hydrophobic adsorptive interactions become important [18]. ERL is a heterocyclic compound with a pKa of 5.42 and Kow 3.37 [2], structurally ERL could interplay with surface O-containing groups of CNT through hydrogen bonding and π-hydrogen bonding interactions [13] besides π-π interactions between benzene rings of ERL with CNT surface.

Moreover, this trend could be attributed to augmentation of f-CNTs dispersibility. In this study, the oxygenated or nitrogenated groups in the CNT surface increased the electrokinetic potential, from -12.7 ±1.4 mV for pristine-CNT to -34.7± 0.09 mV for CNT-COOH and-26.7±0.3 mV for CNT-NH_2, which increased the colloidal stability (fig. 7), and consequently more effective specific sorption area were exposed. In the contrary case, when CNTs dispersion was not stable, because CNTs were prone to aggregation, such as pristine-CNTs, the final effect was the reduction of available area for sorption of organic compounds [13].

Table 1: Fitting results of isotherms for sorption of ERL on the surface of three modified CNTs

Langmuir isotherm						
CNT	**q_e exp. (mg/g)**	**K_L (L/g)**	**k_a (L/mg)**	**q_m (mg/g) (K_L/k_a)**	**R^2**	**P-value**
Pristine	102.07	2.00e-3	-1.00e-3	-2.00	0.35	0.02
COOH	636.04	2.58	1.00e-3	2081.60	0.67	2.13e-4
NH_2	826.76	69.37	0.08	899.44	0.97	3.48e-11
Freundlich isotherm						
CNT		K_f ($mg^{1-n}L^n/g$)	1/n	n	R^2	P-value
Pristine		4.43e-15	2.88	0.35	0.52	2.52e-3
COOH		108.83	0.75	1.34	0.90	8.55e-8
NH_2		277300.08	0.23	4.35	0.33	2.53e-2

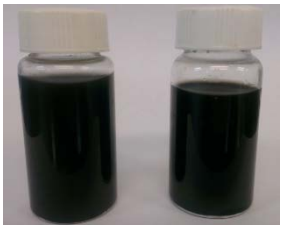
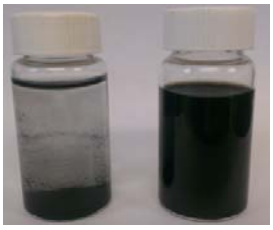
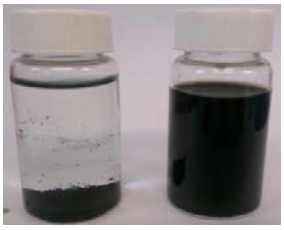
A.  B.  C. 

Fig. 7: Photographs of pristine-CNT (left) and CNT-COOH (right) suspensions in deionized water. After A) two hours B) 24 h and C) two months of resting

The colloidal stability of CNT-NH_2 was different from oxidized and pristine-CNTs, they settled at>24 h, but when they absorbed ERL they settled faster (<24 h), suggesting a change in the repulsive forces. This behavior is favorable because the ERL: CNT-NH_2 complex can be removed by sedimentation from wastewater system.

CONCLUSION

It was found that CNT-COOH and CNT-NH_2 has high adsorption capacity of ERL. The experimental data showed that ERL has the different behavior of adsorption depend on the nature of CNT surface. At the present study was observed the adsorption of ERL followed the Langmuir model for CNT-NH_2 while for CNT-COOH the best model was Freundlich. Pristine-CNT did not fit any models. The results indicated the potential of CNT to removal this antineoplastic drug from hospital wastewater. The loss of dispersibility of ERL: CNT-NH_2 complex has environmental significance because make easy its separation.

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CONFLICT OF INTERESTS

The authors have declared not conflict of interest.

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