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Adsorption behavior of epirubicin hydrochloride on carboxylated carbon nanotubes

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ABSTRACT

The aim of this study was to understand the interaction between carboxylated carbon nanotubes (c-CNTs) and anticancer agents and evaluate the drug-loading ability of c-CNTs. We prepared carboxylated multi-walled carbon nanotubes (c-MWNTs) with nitric acid treatment, then evaluated the adsorption ability of c-MWNTs as adsorbents for loading of the anticancer drug, epirubicin hydrochloride (EPI), and investigated the adsorption behavior of EPI on c-MWNTs. Unmodified multi-walled carbon nanotubes (MWNTs) and single-walled carbon nanotubes (SWNTs) were included as comparative adsorbents. The results showed that carbon nanotubes were able to form supramolecular complexes with EPI via π–π stacking and possessed favorable loading properties as drug carriers. The Freundilich adsorption model was successfully employed to describe the adsorption process. Because of the high surface area and hydrogen bonding, c-MWNTs' adsorption efficiency was the highest and the most stable and their drug-loading capacity was superior to that of MWNTs. With the increase of pH, the adsorption capacity of EPI on the c-MWNTs increased. Low-temperature facilitated the adsorption. More rapid EPI adsorption rate and higher drug-loading ability were observed from c-MWNTs with smaller diameter. Moreover, the adsorption kinetics of EPI on c-MWNTs could be well depicted by using the pseudo-second-order kinetic model.

1. Introduction

Carbon nanotubes (CNTs) are described as hollow cylinders formed by rolling single layer (single-walled CNTs; SWNTs) (Bethune et al., 1993; Iijima and Ichihashi, 1993) or multiple layers (multi-walled CNTs; MWNTs) (Iijima, 1991) of graphene sheets into seamless cylinders. Since their discovery by Iijima, CNTs have attracted great interest because of their intriguing properties and unique structure. They have also been considered to be one of the promising nanomaterials. In recent years, it has been demonstrated that CNTs can not only be loaded with drugs (Feazell et al., 2007; Kam et al., 2005; Murakami et al., 2004; Pastorin et al., 2006; Wu et al., 2005), nucleic acids and peptides (Pantarotto et al., 2004a,b) by forming stable covalent bonds or supramolecular assemblies based on noncovalent interactions, but also have capacity to penetrate into the cells to promote the cellular uptake of therapeutic molecules (Bianco et al., 2005a), which has offered new opportunities for their applications in nanobiotechnology and nanomedicine. Although most of the existing anticancer drugs are very potent small molecules, their efficacy is constrained not only by their systemic toxicity and narrow therapeutic window but also as a result of drug resistance and limited cellular entry. For this reason, the development of efficient delivery systems with the ability to enhance cellular uptake of existing potent drugs is needed. The high aspect ratio of CNTs offers great advantages over existing delivery vectors, because the high surface area provides multiple attachment sites for drugs (Ali-Boucetta et al., 2008). In addition, many oxygen-containing groups, mainly carboxyl and hydroxyl, have been found to decorate the surface of CNTs oxidized with strong acids. The presence of oxygen-containing groups can not only increase their dispersibility in aqueous solutions but can also offer the probability of introducing more than one function on the same tube, so that targeting molecules, contrast agents, drugs, or reporter molecules can be delivered at the same time (Bianco et al., 2005b). Possessing excellent properties mentioned above, CNTs hold great promise as

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Table 1
Selected parameters of carbon nanotubes.

Adsorbents	Diameter[a] (nm)	Length[a] (μm)	Purity[a] (%)	Ash[a] (%)	Special surface area[a] (m^2/g)	Amorphous carbon[a] (%)
MWNT-1020	10–20	5–15	>95	<0.2	40–300	<3
MWNT-4060	40–60	5–15	>95	<0.2	40–300	<3
SWNTs	<2	5–15	>90	<2	>400	<5

[a] Supplied by the manufacturer.

nanovectors for delivery of a variety of therapeutic and diagnostic agents.

Epirubicin hydrochloride (EPI), an analog of doxorubicin hydrochloride, is an anthracycline cytostatic antibiotic with the wide antineoplastic spectrum and high efficiency. However, EPI can result in severe suppression of hematopoiesis and cardiac toxicity. Current studies have suggested that the main method for minimizing the side effects of EPI is to apply drug carrier that can change the biological distribution of EPI and increase its concentration in the local tumor.

The high efficiency and target effect of CNTs for anticancer agent delivery have been demonstrated (Kam et al., 2005). For example, Liu et al. (2009) prepared water-soluble SWNTs functionalised with poly (ethylene glycol) could be loaded with the anticancer drug doxorubicin via π–π stacking interactions, the results of *in vitro* toxicity test suggested that conjugated with targeting molecular ligands, the SWNT–DOX complexes afforded a significantly enhanced therapeutic efficacy and a marked reduction in toxicity compared with free DOX and DOXIL. Heister and Neves (2009) established a method for the triple functionalisation of oxidized SWNTs with doxorubicin, a monoclonal antibody, and a fluorescent marker, which allowed for the targeted delivery of the anti-cancer drug to cancer cells and the visualization of the cellular uptake of SWNTs by confocal microscopy. They found that the complex was efficiently taken up by cancer cells.

Although several studies have reported on the effects of *in vivo* or *in vitro* antitumor activity and tumor targeting of drug–CNTs complex based on the adsorption, very little is known about the adsorption behavior of anticancer drug on CNTs. Analysis for the interaction between anticancer drugs and CNTs serves as the basis for the establishment of transport system. Especially, carboxylated carbon nanotubes (c-CNTs), derivatized with carboxylate groups, exhibit excellent biocompatibility and can be multiply modified to facilitate targeting therapy of cancer. With the increasing application of c-CNTs as anticancer drug delivery system, it becomes necessary to understand the interaction between c-CNTs and anticancer agents and evaluate the drug-loading ability of c-CNTs. In this paper, we prepared carboxylated multi-walled carbon nanotubes (c-MWNTs) with nitric acid treatment and evaluated its ability for loading of the anticancer drug EPI. Furthermore, we systematically investigated the adsorption behavior and mechanism of EPI on c-MWNTs, and elucidated predominant factors influencing drug adsorption. The results of this study were expected to provide a theoretic basis and new directions for preparation of efficient drug carriers.

2. Materials and methods

2.1. Materials

EPI was from Shandong New Time Pharmaceutical Co., Ltd., China. Tris (hydroxymethyl)-aminomethane was purchased from Sinopharm Chemical Reagent Co., Ltd., China. The chemicals were all analytical reagent (A.R.) grade and all solutions were prepared with distilled water. Three kinds of carbon nanotubes with different structures, namely MWNT-1020, MWNT-4060 and SWNTs, were obtained from Shenzhen Nanotechnologies Port Co., Ltd., China. Selected parameters of these carbon nanotubes are listed in Table 1.

2.2. Carboxylation and characterization of MWNTs

0.514 g of raw MWNTs was added to a 100 mL of the concentrated nitric acid, the mixture was sonicated in a water bath for 0.5 h at room temperature and was then dumped into a 250 mL round bottom flask equipped with a condenser and the dispersion was refluxed under magnetic stirring at 140 °C for 10 h. After that, the resulting dispersion was diluted in water and filtered. The resulting solid was washed up to neutral pH, and the sample was dried in vacuum at 65 °C overnight (sample c-MWNTs).

The treated MWNTs samples were characterized with 8400 s FTIR spectrometer (Shimadzu Corporation, Japan), and their morphologies were examined by S-3000 scanning electron microscopy (SEM, Hitachi Corporation, Japan).

2.3. Adsorption experiments

For kinetic study, 300 μg/mL EPI solutions (50.00 mL, pH 7.0) were respectively agitated with a certain amount of CNTs (MWNTs: 0.125 g; SWNTs: 0.05 g; c-MWNTs: 0.12 g) at 25 °C, samples were taken at different intervals until the adsorption reached equilibrium.

Static equilibrium adsorption experiments were performed using screw-capped glass centrifuge tubes as batch reactor systems. Each tube containing a certain amount of CNTs (MWNTs: 0.15 g; SWNTs: 0.005 g; c-MWNTs: 0.01 g) was filled with 5.00 mL EPI solution of different concentrations. All tubes were immediately sealed and were then mechanically shaken for a certain time in a water bath at 25 °C for 240 min.

Separate sets of experiments were conducted to test the effects of pH, temperature and diameter of CNTs on EPI adsorption. The influence of pH on EPI adsorption was studied by adjusting EPI solutions (60 μg/mL) to different pH values (from 5.0 to 9.0, adjusted with Tris–HCl buffer solution) using PHS-25 exact pH meter (Shanghai WEI YE Instrument Factory, China) and agitating 50.00 mL of EPI solution with 0.02 g of c-MWNTs at 25 °C for 240 min. The effect of temperature on EPI adsorption was carried out in 50.00 mL of EPI solutions (60 μg/mL, pH 7.0) with 0.02 g of c-MWNTs until equilibrium was established. The effect of diameter of CNTs on EPI adsorption was carried out in 50.00 mL of EPI solutions (300 μg/mL) with a certain amount of two types of c-MWNTs (c-MWNTs1020: 0.12 g; c-MWNTs4060: 0.125 g) at 25 °C until the adsorption reached equilibrium.

After the predetermined time was completed, the samples were centrifuged (8000 rpm for 4 min) and the supernatant solution was then analyzed by UV1800 UV–Vis spectrophotometer (Shimadzu Corporation, Japan). The amount of EPI adsorbed by CNTs was calculated by the following equation:

$$q_t = \frac{(C_0 - C_t)V}{m} \quad (1)$$

where q_t is the amount of EPI adsorbed on CNTs (mg/g), C_0 is the initial concentration of EPI (mg/L), C_t is the instantaneous content

of EPI at time t (mg/L), V is the solution volume (L), and m is the mass of CNTs used in the adsorption tests (g).

2.4. EPI release from c-MWNTs

The drug release experiment from CNTs was performed according to the method reported by Zhang et al. (2009). The suspensions of the EPI loaded c-MWNTs (20 mg) were allowed to stand at 37 °C in pH value of 7.4 and 5.5 phosphate-buffered saline (PBS) solutions (50 mL). The released medium (1 mL) was withdrew at predetermined time intervals and replaced with equal volumes of fresh medium. The release amount of EPI was detected by UV–Vis spectrophotometry above described.

3. Results and discussion

3.1. Dispersion stability of CNT suspensions

Pristine CNTs have a strong tendency to agglomerate due to their high aspect ratio (Rastogi et al., 2008) and insolubility (Klumpp et al., 2006). However, the grafting of chemical functionalities on the CNTs surface, such as carboxyl and hydroxyl, can improve the solubility or dispersibility of CNTs in water (Datsyuk et al., 2008). Fig. 1 shows the dispersion stability of CNT suspensions in water. In contrast, the untreated MWNTs and SWNTs rapidly and completely precipitated in 30 min to the bottom of the vials and exhibited more precipitate one week later, whereas the c-MWNTs well dispersed in the deionized water for at least one week. The results indicate that the suspension of c-MWNTs is more stable and nitric acid treatment applied is found to improve the dispersion stability of CNTs in water.

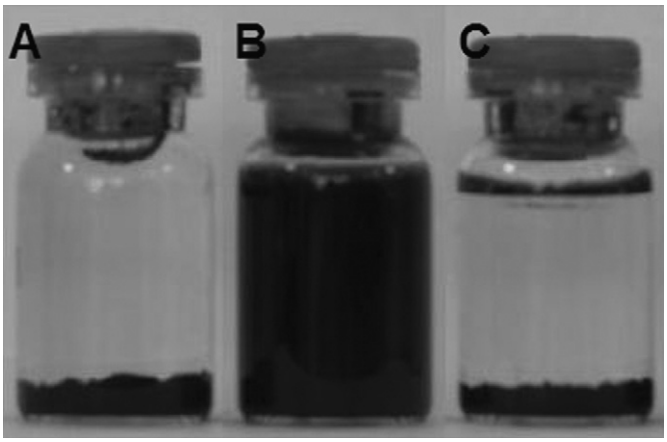

Fig. 1. Dispersion state of CNTs in water: (A) MWNTs, (B) c-MWNTs and (C) SWNTs. The pictures were taken one week after the solution had been sonicated for 5 min.

3.2. SEM image analysis of CNTs

Fig. 2 presents SEM microphotographs of pristine MWNTs (Fig. 2A), acid-modified MWNTs (Fig. 2B) and pristine SWNTs (Fig. 2C). All of the untreated MWNTs and SWNTs were curled and entangled. With oxidation (Fig. 2B), the MWNTs were seen with more open ends (bright spots on the image) with granular surface

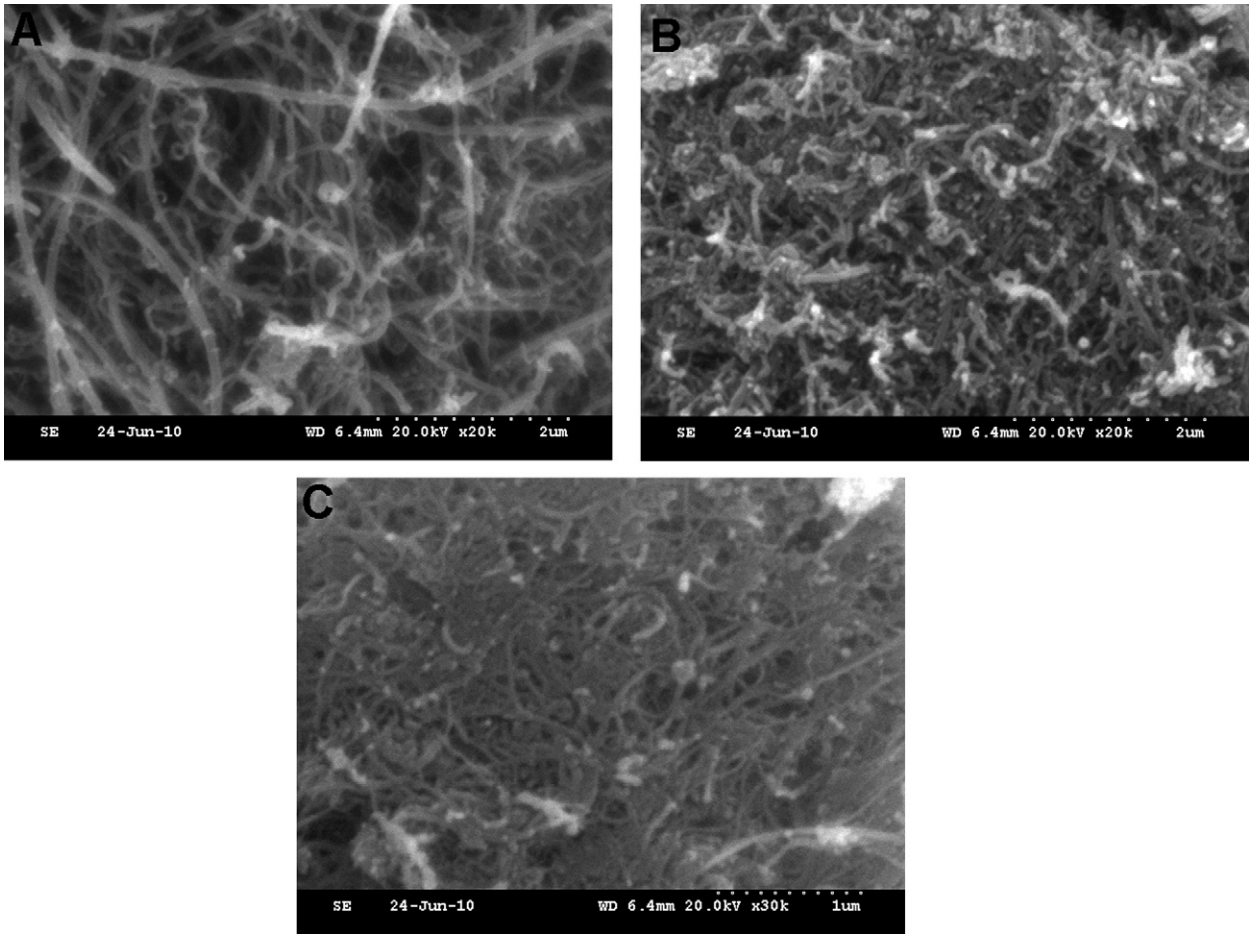

Fig. 2. SEM images of (A) MWNTs (20,000×), (B) c-MWNTs (20,000×) and (C) SWNTs (30,000×).

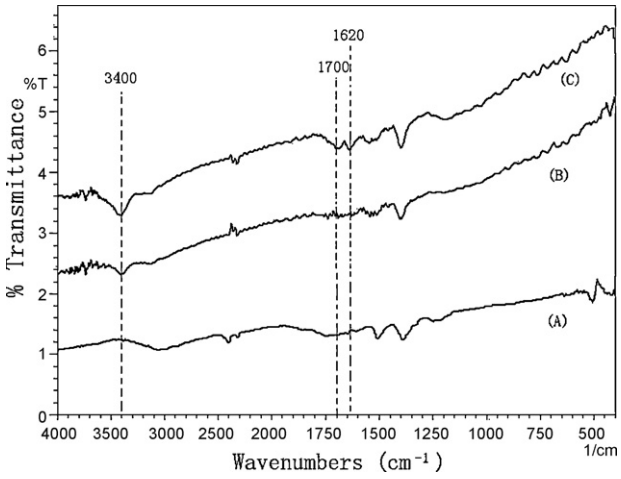

Fig. 3. FTIR spectra of: (A) SWNTs, (B) MWNTs, and (C) c-MWNTs.

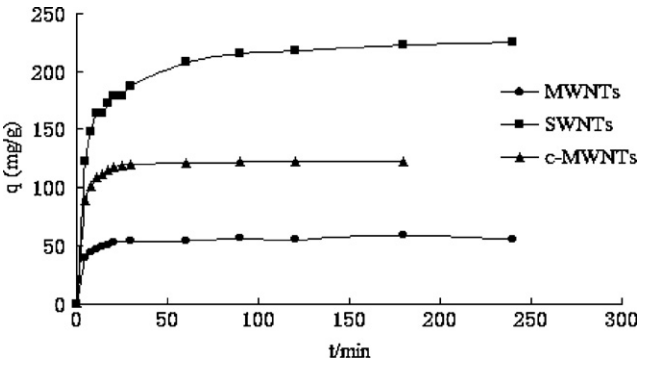

Fig. 4. Kinetic curves for adsorption of EPI on different CNTs (EPI concentration: 300 μg/mL; MWNTs dose: 0.125 g/50.00 mL; SWNTs dose: 0.05 g/50.00 mL; c-MWNTs dose: 0.12 g/50.00 mL; temperature: 25 °C; pH: 7.0).

and joining tubes together. In addition, after oxidation with nitric acid, some bundles appeared exfoliated and the c-MWNTs seemed to be shorter length than pristine MWNTs. The above observations indicate that after nitric acid treatment, the MWNTs become reactive at the ends as well as at the sidewalls (Kim et al., 2006). A large amount of oxygen-containing groups modified on those defect sites can support the stability of c-MWNTs in polar media (Datsyuk et al., 2008).

3.3. IR analysis of CNTs

Strong acid treatment has been used for purification or functionalisation of CNTs (Hu et al., 2003; Marshall et al., 2006), these acid treatments produce carboxylic acid groups and hydroxyl groups on surface of CNTs via oxidations of double bonds in the graphene wall (Shieh et al., 2007). Fig. 3 shows the surface groups on CNTs analyzed by FTIR. Comparing with spectrum A and B, several significant changes of band in spectrum C are attributed to the introduction of carboxylic acid groups by acid oxidizing, including the appearance of C=O stretching band at 1700 cm^{-1}, COO^- asymmetric stretching band at 1620 cm^{-1} and the increase of O–H stretching band at 3400 cm^{-1} (Sun et al., 2001). The peak at around 3500 cm^{-1} (low intensity) of MWNTs corresponding to –OH stretching might be caused by moisture in the samples. Apparently, carboxylic acid groups are successfully introduced by acid oxidizing.

3.4. Adsorption kinetics

The adsorption kinetics of EPI adsorbed on different CNTs are presented in Fig. 4. EPI demonstrated relatively fast adsorption kinetics on three types of CNTs. The adsorption rates of EPI were very rapid at the initial stages of adsorption. After a very rapid adsorption, EPI uptake rates on MWNTs, c-MWNTs and SWNTs slowly declined with lapse of time and reached equilibrium values at about 30 min, 30 min and 90 min, respectively. In addition, no significant desorption was observed.

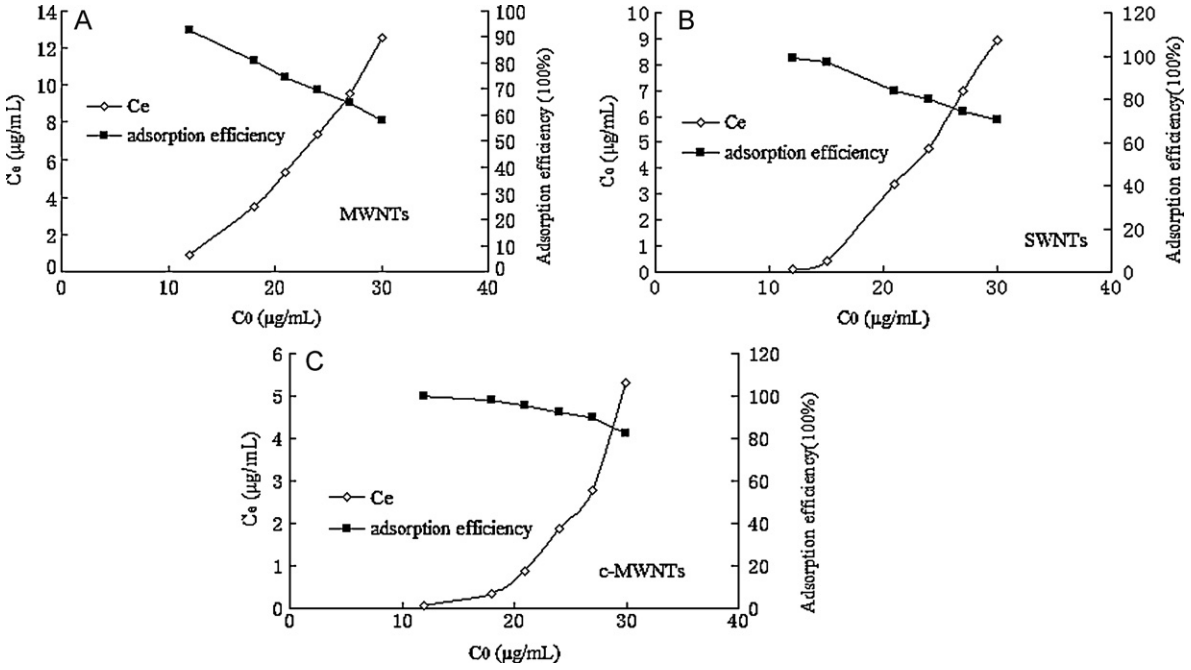

Fig. 5. Comparison of adsorption efficiency of EPI on CNTs (C_0 represents the initial aqueous EPI concentration; C_e represents the aqueous EPI concentration at equilibrium; MWNTs dose: 0.15 g/5.00 mL; SWNTs dose: 0.005 g/5.00 mL; c-MWNTs dose: 0.01 g/5.00 mL; temperature: 25 °C; pH: 7.0; equilibrium time: 240 min).

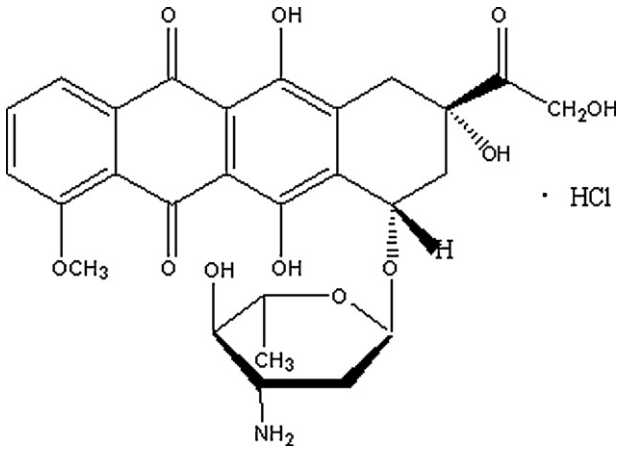

Fig. 6. Molecular structure of epirubicin hydrochloride.

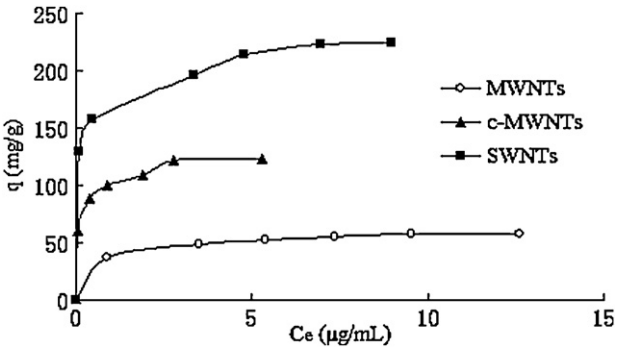

Fig. 7. Adsorption isotherm of EPI on different CNTs (MWNTs dose: 0.15 g/5.00 mL; SWNTs dose: 0.005 g/5.00 mL; c-MWNTs dose: 0.01 g/5.00 mL; temperature: 25 °C; pH: 7.0; equilibrium time: 240 min).

3.5. Adsorption efficiency

In order to find the ideal CNTs with the best adsorption performance, we compared the adsorption efficiency of EPI on MWNTs, SWNTs and c-MWNTs by static equilibrium adsorption experiments. As can be seen from Fig. 5, all tested CNTs had great abilities to adsorb EPI, the highest adsorption efficiency was obtained when the EPI concentration was lowest, along with the increase of EPI dosage, the adsorption efficiency of EPI decreased from 92.80 to 58.05% on MWNTs, from 99.31 to 70.21% on SWNTs and from 99.61 to 82.32% on c-MWNTs, respectively. The test results indicate, compared with MWNTs and SWNTs, c-MWNTs exhibit the highest adsorption efficiency, which could also stay stable with the increasing EPI concentration. It may be explained by the following two reasons: on the one hand, pristine CNTs easily adhere to each other and form bundles due to strong van der Waals interactions, which can obviously decrease their surface area. However, through the oxidation treatment, the pristine CNTs can be effectively purified and shortened, additionally, oxygen-containing groups, mainly carboxyl and hydroxyl, have been found to decorate its surface. The presence of oxygen-containing groups facilitates the exfoliation of CNT bundles, and increases the solubility in polar media (Chen et al., 2001; Han et al., 2007; Liu et al., 1998). More adsorption sites are therefore available and specific surface area of c-MWNTs is higher than that of pristine MWNTs, which may contribute to the enhanced adsorption of EPI on c-MWNTs. On the other hand, EPI (Fig. 6) has OH groups and amino group that can interact with c-MWNTs in other ways. Lin and Xing (2008) have observed that adsorption of phenolics to CNTs with carboxyl and hydroxyl was greatly enhanced by OH substitution. Similar observations were noted by Chen et al. (2007), they suggested that hydrogen bonding between the adsorbate OH group and the adsorbent oxygen-containing groups was one of the possible mechanisms that was proposed to explain the influence of OH substitution on organic compound adsorption by MWNTs. More hydrogen and oxygen contents were detected on the c-MWNTs, indicating that hydrogen bonding might be significant between EPI and the functional groups on the c-MWNTs. The introduction of oxygen-containing groups might improve adsorption stability and increase adsorption capacity of CNTs for EPI.

3.6. Adsorption isotherm

The adsorption isotherm indicates the relationship between equilibrium adsorption capacity and equilibrium concentration at constant temperature. The adsorption isotherms of EPI on three types of CNTs at 25 °C are shown in Fig. 7. In general, the equilibrium adsorption capacity increased with the increment of equilibrium concentration, but the equilibrium adsorption capacity did not increase anymore when the initial concentration reached a certain value. Adsorption capacity increased as SWNTs > c-MWNTs > MWNTs. Additionally, adsorption of EPI to the c-MWNTs was considerably stronger than adsorption to the untreated MWNTs.

Chen et al. (2001) examined the noncovalent sidewall functionalisation of single-walled carbon nanotubes for protein immobilization, the polycyclic aromatic hydrocarbons were found to interact strongly with basal plane of carbon nanotubes via π-stacking. Great adsorption capacity of EPI on all of the CNTs was observed in this study, this phenomenon is mainly attributed to the effective π–π stacking interaction between CNTs and three planar and aromatic hydroxyanthraquinonic structures of EPI (Fig. 8). As can be seen from the test, CNTs are indeed highly effective drug vectors. CNTs tend to aggregate together as bundles because of van der Waals interactions (Zhao et al., 2002). Theoretically, four types of adsorption sites (Fig. 9) are available for adsorption on CNT bundles or aggregates, including surface, inner pores, groove area, and interstitial pores (Foldvari and Bagonluri, 2008). However, according to Pan and Xing (2008), external surface and groove area are generally available for adsorption, but the interstitial and inner pores are not. On the one hand, inner pores of CNTs are not available for adsorption because of the amorphous carbon and metals on both end (Yang et al., 2006), the blocked inner pores can be opened up by acid treatment using nitric acid (Gotovac et al., 2007). On the other hand, Gotovac et al. (2007) find that the external surface of SWNTs is the main area for naphthalene adsorption, but the inner pore sites are not due to the dimensional restriction. The reason for the unavailable interstitial sites is that the organic molecules are too large to fit into this area (Hilding and Grulke, 2004). EPI molecular

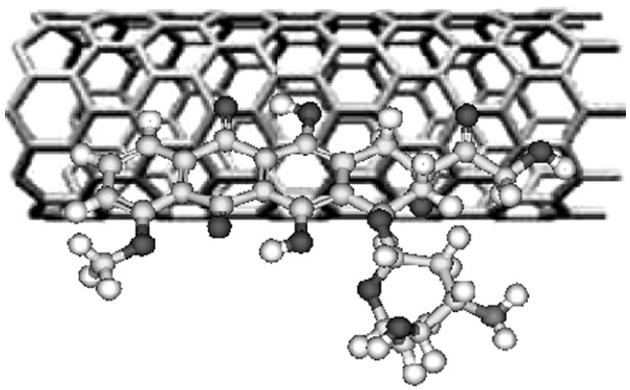

Fig. 8. Schematic representation of EPI π-stacking onto carbon nanotubes.

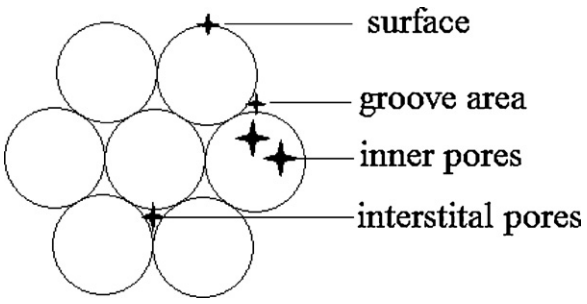

Fig. 9. Schematic representation of adsorption sites available for adsorption on CNT bundles.

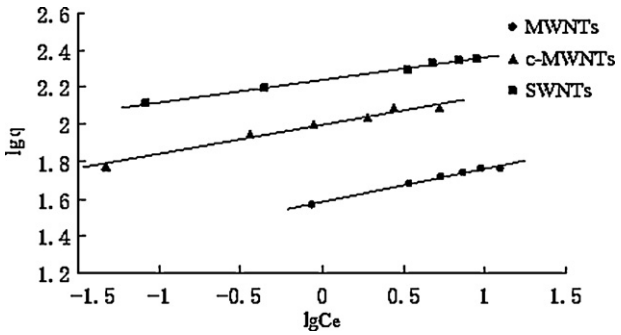

Fig. 10. Freundilich isotherm for EPI adsorption on different CNTs at 25 °C.

structure is relatively larger, thus, we infer that external surface area is the most important factor dominating EPI adsorption on CNTs. As noted in Table 1, MWNTs surface areas were in the range of 40–300 m^2/g and were generally lower than that of SWNTs (more than 400 m^2/g), EPI adsorption was much higher on SWNTs than on MWNTs, which was consistent with the surface area of the adsorbent. Moreover, the stronger EPI adsorption on c-MWNTs than on MWNTs can be well explained by the difference in accessibility of the adsorbent surface area. The interstitial space of MWNTs aggregates might exclude large EPI molecules from accessing the surface area. In contrast, more adsorption sites of c-MWNTs were available due to their enhanced dispersibility in water, therefore resulting in the higher adsorption capacity of c-MWNTs. However, the adsorption capacity of c-MWNTs was still lower than that of SWNTs, the reason could be that the specific surface area of c-MWNTs is smaller than that of SWNTs. It is undoubted that c-MWNTs with the excellent dispersibility, increased adsorption capacity and more stable adsorption efficiency are promising vehicles for anticancer agents. Consequently, the following experiments attempted to determine the effects of various conditions on adsorption of EPI by c-MWNTs.

Two different models were applied to study the adsorption isotherm. The Langmuir equation is valid for monolayer adsorption on a surface with a finite number of identical sites and is expressed as:

$$\frac{1}{q} = \frac{1}{q_0} + \frac{1}{q_0 K_L C_e} \quad (2)$$

where q is the solid-phase concentration (mg/g), C_e is equilibrium solution-phase concentration (mg/L), q_0 is the maximum adsorption capacity and K_L is the Langmuir constant related to affinity of the binding sites.

Freundlich model is an empirical equation based on adsorption on a heterogeneous surface or surfaces supporting sites of varied affinities. It is assumed that the stronger binding sites are occupied first and that the binding strength decreases with the increasing degree of site occupation. The isotherm is expressed as:

$$\lg q = \lg K_F + \frac{1}{n} \lg C_e \quad (3)$$

where q is the amount of adsorbate adsorbed per unit mass of the adsorbent (mg/g), C_e is the adsorbate concentration at equilibrium (mg/L), K_F and n are Freundlich constants related to adsorption capacity and adsorption intensity of the adsorbent. Additionally, the magnitude of the exponent n gives an indication on the favorability of adsorption. It is generally stated that values of n in the range 2–10 represent good, 1–2 moderately difficult, and less than 1 poor adsorption characteristics.

The equilibrium adsorption data of EPI on CNTs were analyzed by Langmuir and Freundlich models (Fig. 10). All the correlation coefficient, R^2 values and the constants obtained from the models are summarized in Table 2. The correlation coefficients of Freundlich exceeded 0.98, indicating that the Freundlich model gave a better fit than the Langmuir model on the adsorption of EPI using different CNTs. According to K_F, indicating the relative adsorption capacity of the adsorbent, the order of adsorption capacity of CNTs was: SWNTs > c-MWNTs > MWNTs. The results show that the values of the Freundlich exponent n were greater than 2 values, confirming that the adsorption process is favorable and all CNTs are excellent adsorbents for EPI.

Table 2
Freundilich and Langmuir isotherm model constants and correlation coefficients for adsorption of EPI on CNTs at 25 °C.

CNTs	Freundilich isotherm model			Langmuir isotherm model		
	K_F	n	R^2	q_{max}	K_L	R^2
MWNTs	38.07	5.56	0.99	58.82	1.87	0.97
c-MWNTs	99.88	6.36	0.98	112.36	22.25	0.93
SWNTs	174.14	8.40	0.99	208.33	16	0.85

3.7. Effect of pH values on adsorption

The pH value is one of the most important factors for the adsorption process. EPI is known as an amphiphilic weak base ($pK_a = 7.7$) due to its hydrophobic anthracycline and hydrophilic sugar moiety (Gao et al., 2010). In this study, the effect of pH value on the adsorption capacity of EPI on c-MWNTs was investigated and shown in Fig. 11. The range of pH value for EPI adsorption tests was between 5.0 and 9.0. The results show that the loading capacity of EPI on c-MWNTs decreased from 131.3 to 120.8 mg/g as pH value reducing from 9.0 to 5.0. This indicates that the amount of EPI loaded on c-MWNTs is pH-dependent. Three possible interactions between EPI and c-MWNTs are as follows: (a) π–π stacking interaction; (b) hydrophobic interaction and (c) hydrogen bonding interaction between the EPI –OH and the tube surface –COOH group. The first two interactions exist between CNTs and the EPI whereas the final one only exists between c-MWNTs and the EPI because of the

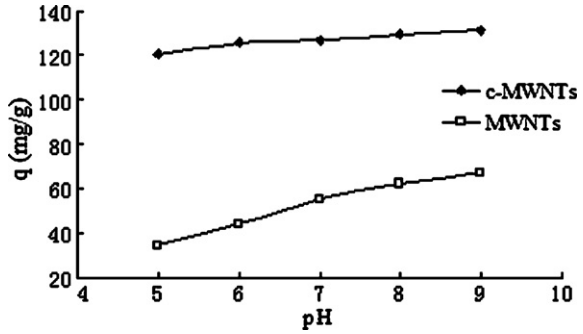

Fig. 11. Effect of the pH values on the adsorption capacity of EPI on CNTs (EPI concentration: 60 μg/mL; c-MWNTs dose: 0.02 g/50.00 mL; MWNTs dose: 0.02 g/50.00 mL; temperature: 25 °C; equilibrium time: 240 min).

introduction of carboxyl groups. Different mechanisms may act simultaneously and respond differently to the change of pH value, thus the prediction of EPI adsorption on c-MWNTs is not straightforward.

We proposed that the most primary mechanism for EPI adsorption on CNTs was π–π interaction between the π-electron-rich aromatic hydroxyanthraquinonic structures of EPI and the π-electron-depleted regions on the graphene surfaces of CNTs (Chen et al., 2008; Liu et al., 2007). When –OH, a strong electron-donating group, dissociates to $–O^-$ at high pH value, the electron-donating strength would be further improved (Chen et al., 2008), which can strengthen the π–π interaction between the EPI and CNTs and thus the adsorption affinity (Lin and Xing, 2008). In addition, the outer surface of individual CNTs can provide hydrophobic sites for organic chemicals (Pan and Xing, 2008). EPI is amphiphilic and its hydrophobic anthracycline can have hydrophobic interaction with hydrophobic sites on CNTs (Liu et al., 2007). The increased hydrophilicity and higher solubility of EPI at lower pH value caused by the increased protonation of $–NH_2$ groups on EPI may reduce the hydrophobic interaction between EPI and CNTs. Compared with MWNTs, c-MWNTs can have hydrogen bonding interaction with EPI. It has been reported that the dissociation of –OH groups on the phenolics may inhibit the formation of hydrogen bonds between phenolic molecules and oxidized CNTs surface (Lin and Xing, 2008). For EPI, when the pH value exceeds its pK_a (7.7), both the –OH group on EPI and the carboxyl group on c-MWNTs would be ionized. Accordingly, the hydrogen-bonding effect should have been impeded, which is unfavorable for adsorption.

The apparent pH value effect on EPI adsorption onto c-MWNTs depends on how the increase in attractive forces (e.g., π–π interaction and hydrophobic interaction) counteracts the decline of certain attractive interaction (e.g., H-bond formation). The result shows that the adsorption capacity was enhanced with the increment of pH value. This indicates that the decrease of the hydrogen-bonding effect was not too strong to balance the increase in π–π stacking and hydrophobic interactions. The amount of EPI loaded on MWNTs with different pH values is also shown in Fig. 11. The interactions between EPI and MWNTs, an adsorbent without carboxyl groups, are π–π stacking and hydrophobic interactions. Therefore, the adsorption capacity of EPI on MWNTs increased much faster than that on c-MWNTs as the increasing pH value.

According to the above analysis, –COOH groups on c-MWNTs are mainly involved in the formation of hydrogen bonds. Although changing the pH value over the range of 5–9 indeed affected the protonation–deprotonation transition of carboxyl groups, the effect of such a transition on hydrogen bonds was not significant. This can be explained by the fact that the hydrogen bonding interaction is between oxygen atoms in –COOH and hydrogen atoms in –OH. Chen et al. (2008) studied the effect of pH value (3–11) on naphthalene adsorption on the carboxylated CNTs. They proposed that the protonation–deprotonation transition of CNTs surface groups such as –COOH had little effect on the adsorptive affinity of nonpolar compounds because the adsorption of naphthalene remained practically constant within the tested pH range. According to Lin and Xing (2008), the increasing adsorption of polar aromatics (e.g., phenolic compounds) to CNTs with increasing pH value may not be due to the change of CNTs properties with pH value. Consequently, we proposed that the specific electronic properties of the EPI functional groups played an important role in the adsorption to c-MWNTs with the changing pH value.

3.8. Effect of temperature on adsorption

The effect of temperature on adsorption capacity of EPI on c-MWNTs is shown in Fig. 12. As can be seen, the adsorption capacity decreased from 131.2 mg/g to 105.6 mg/g with the increment of temperature from 20 °C to 80 °C, and higher temperature is unfavorable to the adsorption process, indicating adsorption is an exothermic process. This result is consistent with previous study which showed that the adsorption of atrazine on SWNTs and MWNTs was exothermic (Yan et al., 2008). Temperature is an important parameter for the adsorption process, thus the room temperature is more appropriate for the preparation of EPI-loaded c-MWNTs.

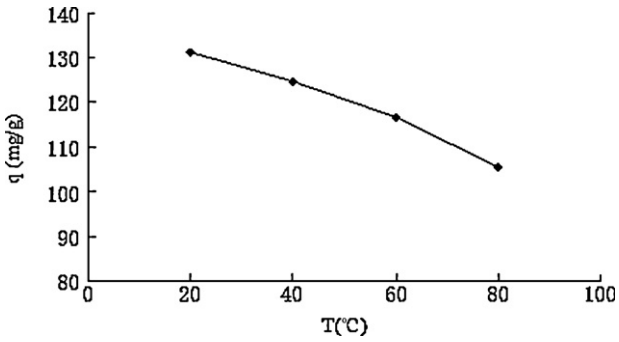

Fig. 12. Effect of the temperature on adsorption capacity of EPI on c-MWNTs (EPI concentration: 60 μg/mL; c-MWNTs dose: 0.02 g/50.00 mL; pH: 7.0; equilibrium time: 240 min).

3.9. Effect of nanotube diameter

The adsorption kinetics of EPI adsorbed on c-MWNTs with two different diameters are given in Fig. 13. EPI demonstrated relatively fast adsorption kinetics on both of them. To further analyze the experimental data, two different models were used to investigate kinetic parameters for the adsorption process. The pseudo-first-order model is described as:

$$q_e - q_t = \lg q_e - \frac{k_1}{2.303} t \tag{4}$$

where k_1 is the equilibrium rate constant for pseudo-first-order; q_e and q_t (mg/g) are the adsorbed amounts at equilibrium and at time t (min), respectively. A plot of $(q_e - q_t)$ versus t should give a straight line, however, it was noticed that the whole experimental data of EPI adsorption onto c-MWNTs did not follow the pseudo-first order model.

The pseudo-second-order model is described as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{5}$$

where q_e and q_t (mg/g) refer to the amount of EPI adsorbed at equilibrium and time t (min), respectively, and k_2 is the rate con-

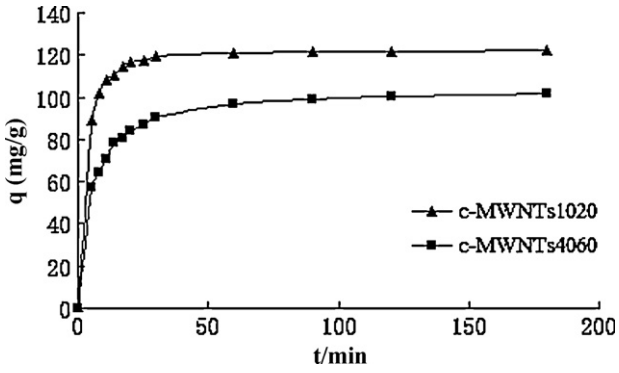

Fig. 13. Kinetic curves for adsorption of EPI on two different CNTs (EPI concentration: 300 μg/mL; c-MWNTs1020 dose: 0.12 g/50 mL; c-MWNTs4060 dose: 0.125 g/50 mL; temperature: 25 °C; pH: 7.0).

Table 3
Pseudo-second-order model constants and correlation coefficients for adsorption of EPI on CNTs at 25 °C.

CNTs	q_e (mg/g)	k_2 (g/mg/min)	R^2
c-MWNTs1020	123.46	5.964×10^{-3}	1
c-MWNTs4060	104.17	2.008×10^{-3}	1

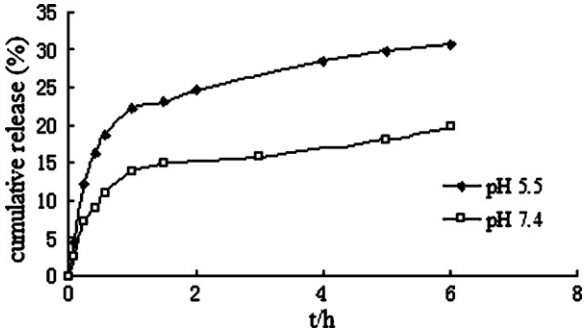

Fig. 14. Release behavior of EPI from c-MWNTs at different pH values (EPI loading content: 121.6 mg/g; EPI-loaded c-MWNTs dose: 0.02 g/50 mL; temperature: 37 °C).

stant. The q_e and k_2 values are calculated from the slope and intercept of the linear plot of t/q_t versus t. The high values of correlation coefficients showed that the data conformed well to pseudo-second-order rate kinetic model (Table 3). This result indicates that the adsorption rate of EPI depends on the concentration of EPI at the adsorbent surface and the absorbance of these absorbed at equilibrium (Sun and Wang, 2006).

As noted in Table 3, the q_e of c-MWNTs1020 was higher than that of c-MWNTs4060, meanwhile, the adsorption rate decreased as c-MWNTs diameter increased from 20 to 60 nm, as indicated by increased equilibration time, because of the decreased surface area. The findings lead us to believe that the overall surface area is the dominant factor that can affect the adsorption capacity and rate of EPI on c-MWNTs. Thus, by choosing c-MWNTs of a specific diameter, one can vary the adsorption rate of drugs and suit different applications.

3.10. In vitro release of EPI

Fig. 14 indicates that the adsorption of EPI on c-MWNTs was reversible. All of the release curves show a rapid release process in the initial stage, and followed by a slow and sustained release process, which seems to continue for a prolonged period of time. Moreover, the amount of EPI released from c-MWNTs is 30.65% after 6 h in the acidic solution, which is almost 1.5 times larger than that in the neutral solution. This result can be mainly attributed to the decreasing π–π stacking and the increasing hydrophilicity of EPI at low pH value. It has been reported that the local pH value of solid tumor (pH < 6.5) was lower than that of normal tissue (pH 7.4) (Liu et al., 2007; Stubbs et al., 2000; Zhang et al., 2009). Therefore, the pH-sensitive release behavior of EPI from c-MWNTs may benefit the tumor treatment.

4. Conclusions

c-MWNTs were prepared with nitric acid treatment and characterized with SEM and FTIR. The results showed that c-MWNTs were successfully introduced with carboxylic acid groups by acid oxidizing and exhibited excellent dispersibility in water. Adsorption tests of anticancer drug EPI on CNTs were carried out. We found that EPI could strongly and rapidly be adsorbed on MWNTs, c-MWNTs and SWNTs, which was mainly attributed to π–π stacking between EPI and the graphene surface of CNTs. The Freundilich adsorption model was successfully employed to describe the adsorption process and the adsorption capacity of EPI was ordered as follows: SWNTs > c-MWNTs > MWNTs. Because of the high surface area and hydrogen bonding, the adsorption efficiency of c-MWNTs was the highest and the most stable, its drug-loading capacity was superior to that of MWNTs. It was confirmed that c-MWNTs were excellent vehicles for loading of EPI. With the increase of pH, the adsorption of EPI on c-MWNTs increased. It was likely that the dissociated –OH in EPI might strengthen the π–π interaction and the increased hydrophobicity of EPI might enhance the hydrophobic interaction between EPI and c-MWNTs at higher pH value. Low temperatures favored the adsorption process, indicating adsorption was an exothermic process. The adsorption kinetics of EPI on c-MWNTs can be well described by the pseudo-second-order kinetic model. More rapid EPI adsorption rate and higher drug-loading ability were observed from smaller diameter c-MWNTs.

Our work established a novel, easy-to-make formulation of a c-MWNTs-EPI complex with extremely high drug loading efficiency. This new complex overcomes the limitations of other carbon nanotube-based systems, carboxylic acid groups introduced onto the walls or ends of the multi-walled carbon nanotubes could facilitate further functionalisation with a targeting group and increase dispersibility of CNTs in water. The mechanism and influence factors for the adsorption of EPI on c-MWNTs were investigated in this paper in order to provide a theoretic basis and new directions for preparation of efficient drug carriers.

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References

Ali-Boucetta, H., Al-Jamal, K.T., McCarthy, D., Prato, M., Bianco, A., Kostarelos, K., 2008. Multiwalled carbon nanotube–doxorubicin supramolecular complexes for cancer therapeutics. Chem. Commun. 4, 459–461.
Bethune, D.S., Klang, C.H., de Vries, M.S., Gorman, G., Savoy, R., Vazquez, J., Beyers, R., 1993. Cobalt-catalysed growth of carbon nanotubes with single-atomic-layer walls. Nature 363, 605–607.
Bianco, A., Kostarelos, K., Partidos, C.D., Prato, M., 2005a. Biomedical applications of functionalised carbon nanotubes. Chem. Commun. 5, 571–577.
Bianco, A., Kostarelos, K., Prato, M., 2005b. Applications of carbon nanotubes in drug delivery. Curr. Opin. Chem. Biol. 9, 674–679.
Chen, R.J., Zhang, Y., Wang, D., Dai, H., 2001. Noncovalent sidewall functionalization of single-walled carbon nanotubes for protein immobilization. J. Am. Chem. Soc. 123, 3838–3839.
Chen, W., Duan, L., Wang, L.L., Zhu, D.Q., 2008. Adsorption of hydroxyl- and amino-substituted aromatics to carbon nanotubes. Environ. Sci. Technol. 42, 6862–6868.
Chen, W., Duan, L., Zhu, D., 2007. Adsorption of polar and nonpolar organic chemicals to carbon nanotubes. Environ. Sci. Technol. 41, 8295–8300.
Datsyuk, V., Kalyva, M., Papagelis, K., Parthenios, J., Tasis, D., Siokou, A., Kallitsis, I., Galiotis, C., 2008. Chemical oxidation of multiwalled carbon nanotubes. Carbon 46, 833–840.
Feazell, R.P., Nakayama-Ratchford, N., Dai, H., Lippard, S.J., 2007. Soluble single-walled carbon nanotubes as longboat delivery systems for platinum (IV) anticancer drug design. J. Am. Chem. Soc. 129, 8438–8439.
Foldvari, M., Bagonluri, M., 2008. Carbon nanotubes as functional excipients for nanomedicines: II. Drug delivery and biocompatibility issues. Nanomedicine: NBM 4, 183–200.
Gao, F., Li, L., Zhang, H.Z., Yang, W.Z., Chen, H.L., Zhou, J., Zhou, Z.M., Wang, Y.S., Cai, Y.Y., Li, X.M., Liu, L.G., Zhang, Q.Q., 2010. Deoxycholic acid modified-carboxymethyl curdlan conjugate as a novel carrier of epirubicin: in vitro and in vivo studies. Int. J. Pharm. 392, 254–262.
Gotovac, S., Song, L., Kanoh, H., Kaneko, K., 2007. Assembly structure control of single wall carbon nanotubes with liquid phase naphthalene adsorption. Colloids Surf., A 300, 117–121.
Han, B., Li, Q.N., Wu, S.W., 2007. Selective adsorption of multi-walled carbon nanotubes with liquiritin and isoliquiritin. Acta Pharmacol. Sin. 11, 1222–1226.
Heister, E., Neves, V., 2009. Triple functionalisation of single-walled carbon nanotubes with doxorubicin, a monoclonal antibody, and a fluorescent marker for targeted cancer therapy. Carbon 47, 2152–2160.

Hilding, J.M., Grulke, E.A., 2004. Heat of adsorption of butane on multiwalled carbon nanotubes. J. Phys. Chem. B 108, 13688–13695.
Hu, H., Zhao, B., Itkis, M.E., Haddon, R.C., 2003. Nitric acid purification of single-walled carbon nanotubes. J. Phys. Chem. B 107, 13838–13842.
Iijima, S., 1991. Helical microtubules of graphitic carbon. Nature 354, 56–58.
Iijima, S., Ichihashi, T., 1993. Single-shell carbon nanotubes of 1-nm diameter. Nature 364, 737–1737.
Kam, N.W.S., O'Connell, M., Wisdom, J.A., Dai, H., 2005. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. Proc. Natl. Acad. Sci. U.S.A. 102, 11600–11605.
Kim, Y.S., Cho, J.H., Ansari, S.G., 2006. Immobilization of avidin on the functionalized carbon nanotubes. Synth. Met. 156, 938–943.
Klumpp, C., Kostarelos, K., Prato, M., Bianco, A., 2006. Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. Biochim. Biophys. Acta 1758, 404–412.
Lin, D., Xing, B., 2008. Adsorption of phenolic compounds by carbon nanotubes: role of aromaticity and substitution of hydroxyl groups. Environ. Sci. Technol. 42, 7254–7259.
Liu, J., Rinzler, A.G., Dai, H., Hafner, J.H., Bradly, R.K., Boul, P.J., 1998. Fullerene pipes. Science 280, 1253–1256.
Liu, Z., Fan, A.C., Rakhra, K., 2009. Supramolecular stacking of doxorubicin on carbon nanotubes for in vitro cancer therapy. Angew. Chem. 121, 7804–7808.
Liu, Z., Sun, X.M., Nakayama-Ratchford, Z., Dai, H.J., 2007. Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery. ACS Nano 1, 50–56.
Marshall, M.W., Popa-Nita, S., Shapter, J.G., 2006. Measurement of functionalised carbon nanotube carboxylic acid groups using a simple chemical process. Carbon 44, 1137–1141.
Murakami, T., Ajima, K., Miyawaki, J., Yudasaka, M., Iijima, S., Shiba, K., 2004. Drug-loaded carbon nanohorns: adsorption and release of dexamethasone in vitro. Mol. Pharm. 1, 399–405.
Pan, B., Xing, B.S., 2008. Adsorption mechanisms of organic chemicals on carbon nanotubes. Environ. Sci. Technol. 42, 9005–9013.
Pantarotto, D., Briand, J.P., Prato, M., Bianco, A., 2004a. Translocation of bioactive peptides across cell membranes by carbon nanotubes. Chem. Commun. 1, 16–17.
Pantarotto, D., Singh, R., McCarthy, D., Erhardt, M., Briand, J.P., Prato, M., 2004b. Functionalized carbon nanotubes for plasmid DNA gene delivery. Angew. Chem. Int. Ed. 43, 5242–5246.
Pastorin, G., Wu, W., Wieckowski, S., 2006. Double functionalisation of carbon nanotubes for multimodal drug delivery. Chem. Commun. 11, 1182–1184.
Rastogi, R., Kaushal, R., Tripathi, S.K., 2008. Comparative study of carbon nanotube dispersion using surfactants. J. Colloid Interface Sci. 328, 421–428.
Shieh, Y.T., Liu, G.L., Wu, H.H., Lee, C.C., 2007. Effects of polarity and pH on the solubility of acid-treated carbon nanotubes in different media. Carbon 45, 1880–1890.
Stubbs, M., Mcsheehy, P.M.J., Griffiths, J.R., Bashford, C.L., 2000. Causes and consequences of tumor acidity and implications for treatment. Mol. Med. Today 6, 15–19.
Sun, S.L., Wang, A.Q., 2006. Adsorption kinetics of Cu (II) ions using N,O-carboxymethyl-chitosan. J. Hazard. Mater. 131, 103–111.
Sun, Y.P., Huang, W.J., Lin, Y., Fu, K.F., Kitaygorodskiy, A., Riddle, L.A., Yu, Y.J., Carroll, D.L., 2001. Soluble dendron-functionalized carbon nanotubes: preparation, characterization, and properties. Chem. Mater. 13, 2864–2869.
Wu, W., Wieckowski, S., Pastorin, G., 2005. Targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes. Angew. Chem. Int. Ed. 44, 6358–6362.
Yang, K., Zhu, L.Z., Xing, B.S., 2006. Adsorption of polycyclic aromatic hydrocarbons by carbon nanomaterials. Environ. Sci. Technol. 40, 1855–1861.
Yan, X.M., Shi, B.Y., Lu, J.J., Feng, C.H., Wang, D.S., Tang, H.X., 2008. Adsorption and desorption of atrazine on carbon nanotubes. J. Colloid Interface Sci. 321, 30–38.
Zhang, X.K., Meng, L.J., Lu, Q.H., Fei, Z.F., Dyson, P.J., 2009. Targeted delivery and controlled release of doxorubicin to cancer cells using modified single wall carbon nanotubes. Biomaterials 30, 6041–6047.
Zhao, J.J., Buldum, A., Han, J., Lu, J.P., 2002. Gas molecule adsorption in carbon nanotubes and nanotube bundles. Nanotechnology 13, 195–200.