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Adsorption and Desorption of Phenanthrene on Carbon Nanotubes in Simulated Gastrointestinal Fluids

Zhenyu Wang,[†] Jian Zhao,[†,‡] Lei Song,[‡] Hamid Mashayekhi,[‡] Benny Chefetz,[§] and Baoshan Xing*[,‡]

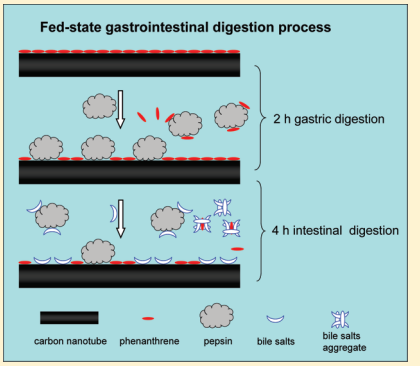
[†]College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China

[‡]Department of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, Massachusetts 01003, United States

[§]Department of Soil and Water Sciences, Hebrew University of Jerusalem, Rehovot 76100, Israel

Supporting Information

ABSTRACT: Adsorption of phenanthrene on carbon nanotubes (CNTs) and bioaccessibility of adsorbed phenanthrene were studied in simulated gastrointestinal fluids. Adsorption of phenanthrene on CNTs was suppressed in pepsin (800 mg/L) solution (gastric) and bile salt (500 and 5000 mg/L) fluids (intestinal). In addition to competitive sorption, pepsin and high-concentration bile salt (5000 mg/L, above critical micelle concentration) solubilized phenanthrene (3 and 30 times of the water solubility, respectively), thus substantially reduced phenanthrene adsorption on CNTs. Pepsin and bile salts also increased the rapidly desorbing phenanthrene fraction from CNTs. The rapidly desorbing phase lasted less than 1 h for all CNTs. Further, 43−69% of phenanthrene was released from CNTs after desorption in the simulated gastric and intestinal fluid at low bile salt concentration while 53−86% was released in the gastric and intestinal fluid at high bile salt concentration. These findings suggest that the release of residual hydrophobic organic compounds from CNTs could be enhanced by biomolecules such as pepsin and bile salts in the digestive tract, thus increasing the bioaccessibility of adsorbed phenanthrene and possibly the overall toxicity of phenanthrene associated CNTs.



INTRODUCTION

With increasing production and use of carbon nanotubes (CNTs), there is an increasing risk that humans could be exposed to ingesting them. There are several possible pathways for CNTs uptake. First, because they are used in health and fitness, food packaging, and biomedicine,[1,2] CNTs are close to and/or could enter the human body directly. Second, CNTs in the environment could be taken up and accumulated by aquatic life (e.g., fish)[3,4] and terrestrial plants (e.g., tomato),[5] thus entering the food chain. Third, normal hand-to-mouth activities of children are important for soil uptake[6] and CNTs in soils could enter the gastrointestinal tract. Moreover, CNTs have been applied as adsorbent media for removal of hydrophobic compounds from drinking water and wastewater treatments.[7,8] For example, CNTs were used as adsorbents in adsorption filters for removal of contaminants from drinking water,[7] which may potentially release CNTs to drinking water. To date, the risks of CNT ingestion are not clearly understood. Limited peroral toxicity studies reported that neither death nor growth or clinical signs of abnormalities were observed for rats (2000 mg/kg of body weight) and mice (1000 mg/kg of body weight).[9,10] Though risks of pure CNT materials could be considered benign based on these toxicity studies, CNTs can be carriers of hydrophobic organic contaminants (HOCs) because of their high adsorption affinity and capacity.[11] HOCs could remain on CNTs in the water after the treatment, and enter the human gastrointestinal tract along with CNTs. Also, polycyclic aromatic hydrocarbons (PAHs), as one type of HOCs, are used as good carbon sources for CNTs synthesis in the arc discharge method,[12] and are important byproduct in the catalytic chemical vapor deposition method with thermal treatment.[13,14] Thus PAHs can be possibly adsorbed on any raw CNTs. Sorption by CNTs can greatly alter the fate, mobility, and bioavailability of PAHs and the environmental risk for both CNTs and PAHs. Therefore, it is necessary to study the adsorption behavior of CNTs and bioaccessibility of adsorbed PAHs on CNTs in gastrointestinal tracts.

In-vitro gastrointestinal tract models have been widely used in estimation of bioavailable HOCs in soils[15,16] and food.[17] However, an in vitro method that simulates the digestion of HOCs (e.g., PAHs)-adsorbed CNTs in the gastrointestinal tract has not yet been reported. It is noted that bioaccessibility of HOCs in soils depends on their solubility in the gastrointestinal fluids,[16] and concentrations of surface-active biomolecules such as bile salts during fasted- and fed-states are different. Thus, we used an in vitro gastrointestinal tract model with low and high concentrations of bile salts in this study according to the human gastrointestinal compositions such as ionic strength and biomolecule

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concentration.[15,18−20] Biomolecules themselves may influence the adsorption of PAHs on CNTs. Competitive adsorptions of PAHs with other organic chemicals such as dissolved organic matter and surfactants were studied and related mechanisms have been explored.[21,22] But it is unknown if the proposed mechanisms in these studies are applicable for biomolecules such as pepsin and bile salts because their structures are different and more complicated. Furthermore, digestive time in the gastrointestinal tract is limited (2 h of gastric digestion and 4 h of intestinal digestion), so desorption of PAHs beyond 6 h is not relevant for human gastrointestinal tract. Phenanthrene is one of the main PAHs byproducts in CNTs synthesis by thermal chemical vapor deposition[14] and a model PAH when studying the adsorption behavior of PAHs.[23] Therefore, the objective of this study was to investigate (i) adsorption of phenanthrene on CNTs in simulated gastrointestinal fluids and the competitive sorption mechanism between phenanthrene and pepsin/bile salts; (ii) desorption kinetics of phenanthrene from CNTs and the effects of pepsin and bile salts; and (iii) bioaccessibility of phenanthrene adsorbed on CNTs in the gastrointestinal tract using an in vitro gastrointestinal model.

MATERIALS AND METHODS

Materials. Radioactive phenanthrene (8.2 μCi/μmol), unlabeled phenanthrene, pepsin, and bile salts were purchased from Sigma-Aldrich Chemical Co. The molecular weight, molecular volume and octanol−water partition coefficient (log K_{ow}) of phenanthrene are 178.2 g/mol, 169.5 Å^3, and 4.57, respectively.[23] The water solubility of phenanthrene at 25 and 37 °C is 1.00 and 1.75 mg/L, respectively, calculated from May and Wasik.[24] Pepsin (600−1800 units/mg protein) is from porcine gastric mucosa and has a molecular weight of 35 kDa. Bile salts are mixtures of 50% sodium cholate (NaC) and 50% sodium deoxycholate (NaDC). Three carbon nanotubes (CNTs) including one single-walled carbon nanotube (SWCNT) and two multiwalled carbon nanotubes (MWCNT10, MWCNT40) were purchased from Shenzhen Nanotech Port Co., China. Their characteristics are listed in Table S1 in the Supporting Information.

Gastrointestinal Fluids Preparation. The gastrointestinal fluids were simulated based on human gastrointestinal compositions such as ionic strength,[18] and pepsin and bile concentrations.[19,20] Briefly, simulated gastric fluid was a NaCl−HCl solution (NaCl 0.1 mol/L, pH 2.0) with 800 mg/L pepsin. Simulated intestinal fluid was a neutral buffered solution (NaCl 0.12 mol/L, Na_2CO_3 0.02 mol/L, pH 7.5) with bile salts at either low (fasted-state) or high (fed-state) concentration (500 or 5000 mg/L). A background solution (0.01 mol/L $CaCl_2$ in distilled water, pH 7.0) was used as the control for comparison with gastrointestinal fluids for the adsorption and desorption experiments. NaN_3 salt was added into all the solutions to avoid the degradation of phenanthrene and the final concentration was 200 mg/L.

Adsorption Experiments. All adsorption isotherms were obtained using a batch equilibration technique in 40-mL vials at 37 °C (body temperature). For phenanthrene adsorption, ^{14}C-labeled and unlabeled phenanthrene were dissolved in methanol as stock solution. The phenanthrene stock solution was diluted sequentially to a series of concentrations using simulated gastric fluid, intestinal fluids (both low and high bile salt concentration), or background solution ($CaCl_2$ 0.01 mol/L). Then 40 mL of each phenanthrene solution was added to a vial that contained 1 mg of CNTs. The vials were then sealed and shaken at 37 °C on a temperature-controlled shaker for 4 days. After equilibration, the vial was centrifuged at 1280g for 15 min, and 1 mL of supernatant was added to 4 mL of Ultima Gold XR cocktail (Perkin-Elmer) for liquid scintillation counting (Beckman LS6500). ^{14}C-labled phenanthrene in simulated gastric and intestinal fluids was measured, which showed that the presence of pepsin and bile salts did not significantly influence the analysis of phenanthrene (Figure S1) and no phenanthrene degradation was observed from HPLC analysis. Therefore, adsorbed phenanthrene by CNTs was calculated directly by mass difference.

For pepsin and bile salts adsorption, pepsin and bile salts were respectively dissolved in NaCl−HCl and NaCl−Na_2CO_3 solutions as described above to obtain various concentrations of pepsin (10−800 mg/L) and bile salts (200−1600 mg/L) solutions. Then 20 mg of SWCNT or 40 mg of MWCNTs were added to each vial which contained 40 mL of pepsin solution while 80 mg of SWCNT or 160 mg of MWCNTs were added to 40 mL of bile salts solution. All vials were shaken at 37 °C for 4 days to reach equilibrium. After centrifugation, the supernatant was filtered through a 0.22-μm Teflon membrane and then analyzed. Pepsin concentrations in the supernatant were determined by a Total Organic Carbon Analyzer (TOC-5000A, Shimadzu) using the NPOC (nonpurgeable organic carbon) method. For bile salts, 0.1 mL of supernatant was added to 3 mL of concentrated sulfuric acid (98%) and let stand for 1 h to form a maximum absorption wavelength at 313 nm, which was then determined by a spectrophotometer (Agilent 8453, USA) and calibrated from the standard curve (r^2 = 0.9992).[25] Adsorbed pepsin or bile salts amounts were calculated directly through the mass difference between the initial and equilibrium concentrations.

Solubility Experiments. Solubilization of phenanthrene by pepsin or bile salts was measured using the method of Yang et al.[22] Briefly, sufficient amounts of ^{14}C-labled and unlabeled phenanthrene in methanol were added to a series of 20-mL vials containing various concentrations of pepsin or bile salts in respective buffer solutions and the maximum phenanthrene concentration in the vial was 50 mg/L. The percentage of methanol in solution of each vial was kept below 0.5% (v/v) to avoid cosolvent effect. The vials were shaken at 37 °C for 4 days and then centrifuged to separate the crystalline and dissolved phenanthrene. Concentrations of phenanthrene in the supernatant were determined by liquid scintillation counting. Critical micelle concentration (CMC) of bile salts in NaCl−Na_2CO_3 buffer solution was obtained by measuring the surface tension of various concentrations of bile salts using a Drop Shape Analysis System (DSA100, Kruss).

Desorption Kinetics Experiment. Desorption of phenanthrene was conducted in the simulated gastric fluid, intestinal fluids at low and high bile salts concentrations, and background solution following a method of Yang and Xing[26] with modifications.[27] Briefly, 230 mL of background solution was added to a 240-mL bottle containing 6 mg of CNTs. Phenanthrene stock solution was then added into each bottle for several times until a stable equilibrium phenanthrene concentration was reached at 0.95−0.98 mg/L (close to water solubility) at 25 °C. After centrifugation, 210 mL of supernatant was removed from each bottle, and amended by the same volume of gastric fluid, intestinal fluids, or background solution immediately. The bottles were resealed and shaken at 37 °C to simulate the gastrointestinal desorption. At different time intervals with a total desorption time of 12 h, the bottles were centrifuged and 0.5 mL supernatant was transferred into scintillation cocktail for liquid scintillation counting. The sum of the sample volumes removed was less than 5% of the total solution volume to avoid any significant change in solid/water ratio.

In-vitro Gastrointestinal Model. A sequential incubation procedure was conducted first in the gastric fluid for 2 h and then in the intestinal fluid (neutral buffered solution with bile salts) for 4 h to simulate the gastrointestinal digestion.[15] After sorption by CNTs (6 mg) in 230 mL of background solution, the equilibrium phenanthrene concentration was 0.95−0.98 mg/L at 25 °C. After centrifugation, 210 mL of supernatant was removed from CNTs, and amended by the same volume of gastric fluid. All solutions were shaken at 37 °C to model the stomach digestive process. After 2 h of incubation, these solutions were centrifuged and 210 mL of supernatants were removed, and amended by the same volume of simulated intestinal fluids with 500 mg/L or 5000 mg/L bile salts to model the intestine digestive process. After 4 h incubation at 37 °C, these solutions were centrifuged. The radioactivity of the supernatants from each process was measured and the concentrations of adsorbed phenanthrene were calculated by mass difference.

Data Analysis. All experiments were run in triplicate. Nonlinear Freundlich and Langmuir models[22] were employed to fit the data of phenanthrene, pepsin, and bile salts adsorption isotherms.

$$q_e = K_f C_e^{\,n}$$

$$q_e = Q^0 C_e/(K_L + C_e)$$

where q_e (mg/kg) is the equilibrium adsorbed concentration of solute; C_e (mg/L) is the equilibrium aqueous concentration; K_f [(mg/kg)/(mg/L)^n] is the Freundlich affinity coefficient, and n is the Freundlich exponential coefficient; Q^0 (mg/kg) is the maximum adsorption capacity; and K_L (mg/L) is the Langmuir affinity coefficient.

Desorption kinetics of phenanthrene from CNTs is described by a first-order, two-compartment model.[28]

$$\frac{S_t}{S_0} = F_{rap}e^{-k_{rap}t} + F_{slow}e^{-k_{slow}t}$$

where S_t and S_0 are the phenanthrene mass adsorbed on CNTs at time t (h) and at the beginning of the desorption procedure, respectively; F_{rap} and F_{slow} are the fractions of phenanthrene present in the rapidly and slowly desorbing CNTs compartments, respectively; k_{rap} (h^{-1}) and k_{slow} (h^{-1}) are the rate constants describing rapid and slow desorption, respectively.

RESULTS AND DISCUSSION

Adsorption of Phenanthrene on Carbon Nanotubes in Gastrointestinal Fluids. All isotherms of phenanthrene were nonlinear (Figure 1, Figure S2) and fit well by Freundlich (Table 1) and Langmuir (Table S2) models. Freundlich fitting results were discussed below because of its higher r_{adj}^2 (adjusted coefficient of determination) values than Langmuir fitting. Nonlinear isotherms suggested that there were different types of surface sites and/or limited available sites on CNTs. For a given CNT, phenanthrene isotherm became more linear in gastric and intestinal fluids than background solution as shown by the higher n values (Table 1). Adsorption of phenanthrene (at two selected concentrations) on CNTs in background, NaCl-HCl, and NaCl-Na_2CO_3 solutions without pepsin and bile salts was studied; the results showed that ionic strength (<0.17 mol/L) and pH (2−7.5) in the simulated fluids did not significantly influence the adsorbed amounts of phenanthrene (Figure S3). Hence, the higher n values of simulated gastric fluid and bile salts solution were mainly caused by pepsin and bile salts. Competitive sorption may have occurred between phenanthrene and pepsin/bile salts, supported by a competitive sorption study between naphthalene and cetylpyridinium on CNTs where n increased in the presence of cetylpyridinium.[22]

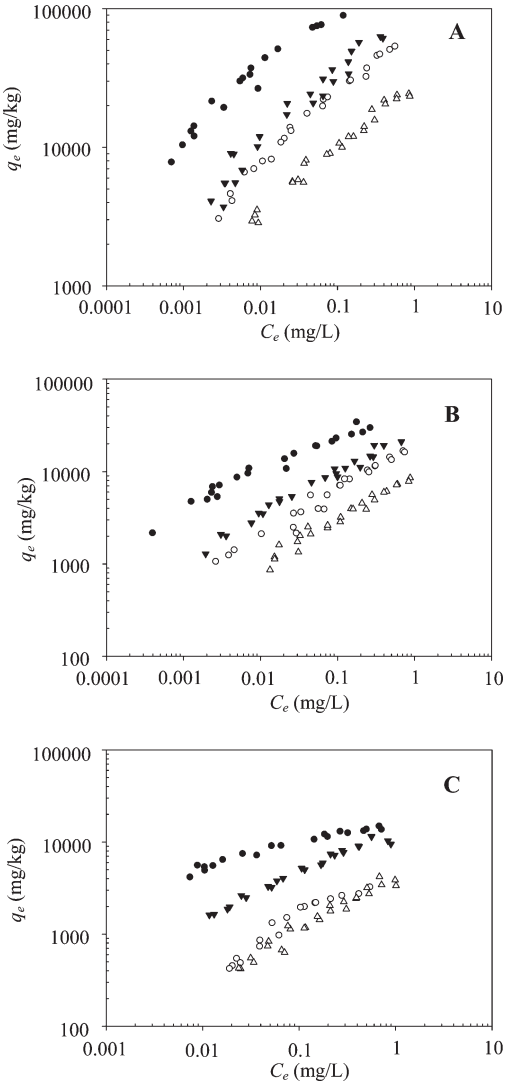

Figure 1. Adsorption of phenanthrene on carbon nanotubes in background solution (BS) (●), simulated gastric fluid (○), simulated intestinal fluids at low (▼) and high (△) bile salts concentrations: (A) SWCNT, (B) MWCNT10, and (C) MWCNT40. The solid phase concentration was on a unit mass basis.

Single point sorption coefficient (K) based on the Freundlich fitting results followed the order of SWCNT > MWCNT10 > MWCNT40 on a unit mass basis for all solutions. However, the order changed to SWCNT > MWCNT40 > MWCNT10 after normalizing by the surface area (K/A_{surf}) (Table 1). Adsorption of pepsin and bile salts on CNTs was also examined (Figure 2A, 2B). For pepsin (calculated at C_e = 800 mg/L) and bile salts (calculated at C_e = 500 mg/L), K/A_{surf} value of MWCNT40 was the highest (Table S3), suggesting that MWCNT40 could be dispersed better than SWCNT and MWCNT10. Therefore, MWCNT40 may have more accessible surfaces for pepsin and bile salts. This may partly account for the difference in the change of K and K/A_{surf} among the three CNTs. Size of adsorbates may be also responsible for the different surface availability of CNTs.

Table 1. Freundlich Model Fitting Results of Isotherms for Phenanthrene Adsorption on Carbon Nanotubes and a First-Order, Two-Compartment Kinetics Model Fitting Results for Phenanthrene Desorption from Carbon Nanotubes in Background Solution, Gastric Fluid, and Intestinal Fluids at Low and High Bile Salts Concentrations

		Freundlich model					first-order, two-compartment kinetics model					
adsorbent	solution	K_f [$\times 10^3$ (mg/kg)/(mg/L)^n]	n	$r_{adj}{}^{2a}$	K^b ($\times 10^3$ L/kg)	K/A_{surf} (L/m^2)	F_{rap}	F_{slow}	F' [f]	K_{rap} (h^{-1})	K_{slow} (h^{-1})	r^2
SWCNT	water[c]	230 ± 16	0.403 ± 0.019	0.964	2880	5.32	0.170 ± 0.004	0.831 ± 0.002	0	10.2	0.00770	0.999
	gastric	72.1 ± 1.6	0.467 ± 0.013	0.989	689	1.27	0.319 ± 0.046	0.681 ± 0.029	0.149	4.24	0.0198	0.961
	intestinal I[d]	104 ± 6	0.477 ± 0.027	0.956	952	1.76	0.272 ± 0.023	0.728 ± 0.065	0.102	2650	0.0155	0.989
	intestinal II[e]	27.6 ± 0.8	0.426 ± 0.019	0.966	314	0.580	0.751 ± 0.089	0.249 ± 0.057	0.581	4370	0.0303	0.987
MWCNT10	water	49.9 ± 2.7	0.342 ± 0.019	0.953	809	2.27	0.228 ± 0.009	0.772 ± 0.006	0	2.64	0.0215	0.998
	gastric	19.4 ± 0.5	0.483 ± 0.019	0.979	173	0.485	0.265 ± 0.010	0.736 ± 0.007	0.037	2.64	0.0402	0.999
	intestinal I	26.6 ± 1.1	0.429 ± 0.022	0.964	298	0.836	0.269 ± 0.018	0.731 ± 0.013	0.041	3.27	0.0515	0.997
	intestinal II	9.02 ± 0.24	0.468 ± 0.019	0.970	85.8	0.240	0.608 ± 0.014	0.391 ± 0.011	0.381	3.53	0.0965	0.999
MWCNT40	water	16.5 ± 0.5	0.245 ± 0.013	0.953	403	4.70	0.308 ± 0.026	0.687 ± 0.019	0	1.64	0.0173	0.990
	gastric	4.61 ± 0.34	0.473 ± 0.034	0.949	42.9	0.499	0.429 ± 0.048	0.571 ± 0.040	0.121	3.01	0.126	0.994
	intestinal I	12.4 ± 0.5	0.407 ± 0.029	0.948	153	1.78	0.387 ± 0.021	0.610 ± 0.016	0.079	1.82	0.0301	0.996
	intestinal II	4.04 ± 0.15	0.536 ± 0.035	0.969	28.8	0.335	0.881 ± 0.034	0.119 ± 0.034	0.573	1.90	0.295	1.00

[a] $r_{adj}{}^2$ is the adjusted coefficient of determination and it is influenced by both the number of data points (m) and the number of fitting parameters (p). $r_{adj}{}^2 = 1 - (m-1)(1-r^2)/(m-p-1)$. [b] K is single point adsorption coefficient calculated on the basis of the Freundlich model when C_e was 0.015 mg/L. [c] Water is the background solution. [d] Intestinal I is the intestinal fluid at low bile salts concentration. [e] Intestinal II is the intestinal fluid at high bile salts concentration. [f] F' is the difference between F_{rap} in the gastrointestinal fluids and F_{rap} in the water for a given CNT.

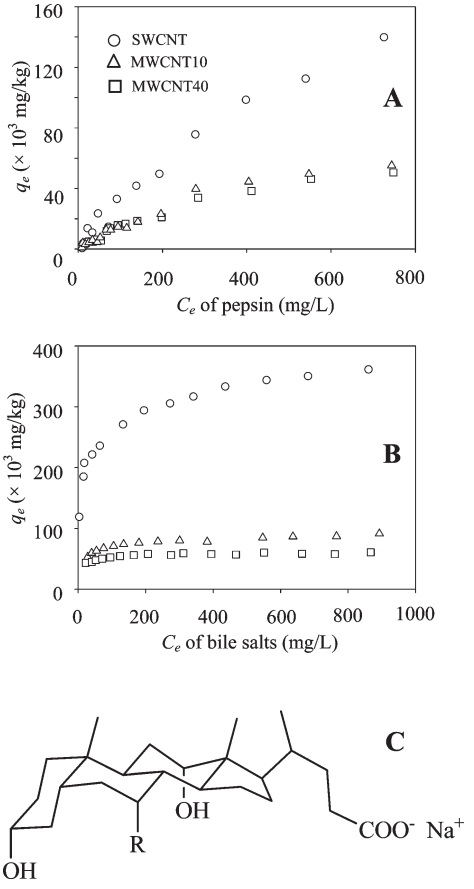

Figure 2. Adsorption isotherms of pepsin and bile salts onto three carbon nanotubes (CNTs) at 37 °C. (A) Pepsin onto CNTs in a NaCl−HCl solution (NaCl 0.1 mol/L, pH 2.0); (B) bile salts onto CNTs in a NaCl−Na_2CO_3 solution (NaCl 0.12 mol/L, Na_2CO_3 0.02 mol/L, pH 7.5); (C) Structure of bile salts molecules. R group is OH for sodium cholate (NaC) and H for sodium deoxycholate (NaDC).

Phenanthrene molecule is small enough to occupy the surface sites in micropores[23] and SWCNTs had the maximum micropore volumes (Table S1). Thus SWCNTs exhibited the highest K/A_{surf} value for phenanthrene. Pepsin (~5 nm) and bile salts (~1.5 nm) could hardly enter the micropores (<2 nm),[29,30] therefore, it is not surprising that MWCNT40 with the lowest micropore volume had the highest K/A_{surf} values for pepsin and bile salts.

For a given CNT, the order of K values was background solution > intestinal fluid at low bile salt concentration > gastric fluid > intestinal fluid at high bile salt concentration. Pepsin and bile salts reduced the adsorption of phenanthrene on CNTs. Hydrophobic effect and $\pi-\pi$ bonding are mainly responsible for phenanthrene adsorption on CNTs.[31] The strength of $\pi-\pi$ bonding could be weakened by the presence of another compound containing benzene rings, and the adsorbed phenanthrene could even be replaced directly.[31] Thus, pepsin molecule could reduce the $\pi-\pi$ bonding strength and lower phenanthrene adsorption because of its aromatic residues (histidine, phenylalanine, tryptophan, and tyrosine) on pepsin surface (11% of total residues) (Figure S4A).[32] However, bile salts, as mixtures of NaC and NaDC, have no double bonds or benzene rings (Figure 2C). Thus $\pi-\pi$ bonding was not responsible for the decrease of phenanthrene adsorption in the intestinal fluids. The strength of the adsorptive interactions can also be affected by the solubility of phenanthrene in different solutions (hydrophobic effect).[31] Pepsin and bile salts both enhanced the solubility of phenanthrene. Solubility of phenanthrene in pepsin solutions showed a Langmuir-like relationship and that in 800 mg/L of pepsin (concentration of pepsin in the gastric fluid) was about 3 times of phenanthrene solubility in water (Figure S4B). As a surfactant, the solubilizing ability of bile salts is highly dependent on the presence of micelle-like aggregates[33] (CMC of about 1000 mg/L) (Figure S5). Phenanthrene solubility only slightly increased in the intestinal fluid at low bile salt concentration (500 mg/L) because this concentration was below its CMC. Hydrophobic region (side) of single bile salts could interact with hydrophobic phenanthrene, thus lowering the interaction between phenanthrene and CNTs. Bile salts at high concentration (5000 mg/L, above CMC) highly solubilized phenanthrene

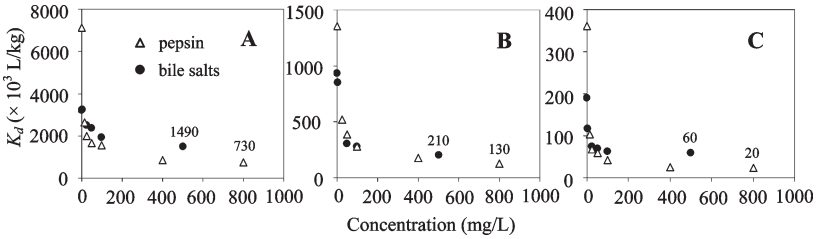

Figure 3. Competitive adsorption between phenanthrene and pepsin (at pH 2.0) or bile salts (at pH 7.5) on SWCNT (A), MWCNT10 (B), and MWCNT40 (C). K_d is the distribution coefficient of phenanthrene at its initial phenanthrene concentration (C_0) of 0.16 mg/L as a function of initial pepsin or bile salts concentrations.

(Figure S5). The saturated solubility in the intestinal fluid at 5000 mg/L was 30 times that of pure water. Calculated from the Langmuir model fitting results of bile salts adsorption by CNTs (Table S4), the equilibrium aqueous concentrations of bile salts were all above 4990 mg/L, far above its CMC, when the initial concentration was 5000 mg/L. Therefore, even after adsorption by CNTs, intestinal fluid with high initial bile salt concentration should still effectively solubilize phenanthrene. Therefore, phenanthrene adsorption reduction in the intestinal fluids could be attributed to the reduced hydrophobic interaction between phenanthrene and CNTs due to enhanced solubilization by bile salts. While in the gastric fluid the reduction was attributed to both surface $\pi-\pi$ bonding and hydrophobic interactions in solution. Intestinal fluid with high bile salts concentration had the highest solubilization and the lowest phenanthrene adsorption.

Competition between phenanthrene and pepsin/bile salts was further examined. In the presence of pepsin/bile salts, adsorption coefficients ($K_d = q_e/C_e$) of phenanthrene at an initial concentration of 0.16 mg/L decreased sharply at low pepsin/bile salts concentrations (below 200 mg/L) and were less obvious with further increasing pepsin/bile salts concentrations (Figure 3). For bile salts, competition would be primarily responsible for the decreased K_d values before 200 mg/L because of limited solubility enhancement (CMC = 1000 mg/L) (Figure S5). However, phenanthrene solubility was also increased at low pepsin concentrations (Figure S4). Thus, both competition and solubility enhancement were responsible for the decreased K_d values, but the exact contribution of each was unknown. At the highest tested pepsin/bile salts concentration, SWCNT, MWCNT10, and MWCNT40 still had the final K_d values of 7.3×10^5, 1.3×10^5, and 2.4×10^4 L/kg for pepsin (800 mg/L) while values were 1.49×10^6, 2.1×10^5, and 6×10^4 L/kg for bile salts (500 mg/L), respectively. These results suggest that some adsorption sites initially occupied by phenanthrene could not be accessed by pepsin/bile salts and that SWCNT had the highest number of these sites. Micropores with high-energy sites were responsible for high phenanthrene adsorption,[23] but they were unable to be occupied by pepsin and bile salts molecules with a size close to or larger than 2 nm. This micropores-dependent result was also supported by the positive relationship between K_d values of phenanthrene (0.16 mg/L) on different CNTs and their micropore volumes (Table S1). K_d values of phenanthrene at an initial concentration of 0.83 mg/L (Figure S6) had the same trend with K_d values of phenanthrene (0.16 mg/L) as discussed above.

Desorption Kinetics of Phenanthrene from CNTs. Adsorption of PAHs on CNTs was reported to be reversible[34] and desorption process in digestive system may result in potential exposure to human body, especially under changing solution chemistry conditions. Phenanthrene was first adsorbed by CNTs in water at 25 °C to simulate adsorption behavior in natural water environments. Desorption kinetics of phenanthrene from CNTs in background solution and gastrointestinal fluids at 37 °C were measured (Figure S7). The data points were fitted well using a first-order, two-compartment model for all solutions. The derived parameters are listed in Table 1.

Desorption of phenanthrene could be treated to occur in a rapidly desorbing phase and then a progressively more slowly desorbing phase, similar to PAHs desorption from sediments.[27] Rapid fraction (F_{rap}) is of more importance in the digestive system because the digestive time is limited (only about 6 h). In comparison with background solution, F_{rap} values in gastrointestinal solutions were much higher for all CNTs, especially in the intestinal fluid at high bile salts concentration (Table 1), indicating that adsorbed phenanthrene on CNTs could have higher potential to release, leading to higher exposure than the background solution. Desorption is kinetically controlled by release from the sorption sites and diffusion through the sorbent to water.[35] Surface competition with phenanthrene (Figure 3) and solubilization of pepsin and bile salts (Figure S4−S5) would help promote the release from the sorption sites and diffusion to gastrointestinal fluids, respectively. Hence, competition and solubilization of pepsin and bile salts were responsible for the high F_{rap} values of the gastrointestinal fluids. Intestinal fluid at low bile salts concentration had much lower F_{rap} values than that at high bile salts concentration due to the lower solubilization enhancement.

As expected, in contrast to F_{rap}, all the F_{slow} values were much smaller in the gastrointestinal fluid than those in the background solution (or water) as the sum of F_{rap} and F_{slow} was 1. Therefore a certain amount of adsorbed phenanthrene (F', the difference between F_{rap} in the gastrointestinal fluids and F_{rap} in the water for a given CNT) should transfer from slow fraction in water to rapid fraction in gastrointestinal fluids (Table 1). Slow desorption of PAHs from sediment was resulted from the entrapment in narrow pores and slow pore diffusion.[27] For CNTs, micropores and narrow pores with high-energy sites could also be responsible for slow desorption in water.[36] Therefore, the higher F_{rap} values in the gastrointestinal solutions could be explained by the following: (i) phenanthrene in micropores was partially released through solubilization by pepsin and bile salts in the simulated fluids (the effect of competitive adsorption would be limited because sizes of pepsin and bile salts are close to or larger than 2 nm); (ii) dispersion of CNTs was increased by pepsin and bile salts thus a fraction of micropores and other narrow pores between individual CNTs could have disappeared. In water, phenanthrene on the CNTs would readily reach the rapid desorption equilibrium (k_{rap}, less than 1 h for all CNTs) while much longer time was required for slow desorption phase, thus desorption would continue in the gastrointestinal tract if CNTs entered the gastrointestinal tract. Therefore, from a toxicological point of view,

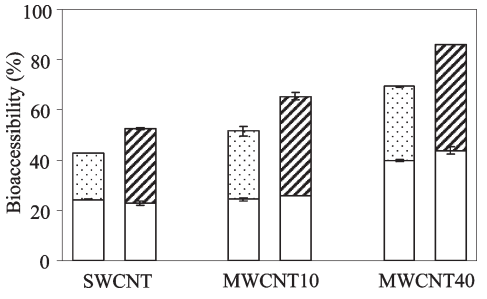

Figure 4. Bioaccessibility of phenanthrene adsorbed on CNTs in the gastric fluid (□) and intestinal fluid at low (dotted bar) or high (striped bar) bile salts concentration using the in-vitro gastrointestinal model. Bioaccessibility (mean ± SD, $n = 3$) was calculated as the percentage of the desorbed phenanthrene amount in the gastric or intestinal fluid to the total adsorbed phenanthrene on CNTs before desorption.

F' is the most important fraction, and SWCNT with the highest F' values and adsorption capacity for all fluids would have higher toxicity potential.

The rapidly desorbing phase lasted less than 1 h for all CNTs according to rate constants of rapid desorption (k_{rap}, h^{-1}). For a given solution, slow desorption times ($1/k_{slow}$) had an order of MWCNT40 > MWCNT10 > SWCNT, in agreement with the order of K_d values at the highest tested concentration of pepsin/bile salts (Figure 3). For MWCNT40 in the intestinal fluid at high bile salts concentration, almost 100% desorption occurred during 4 h ($1/k_{rap} + 1/k_{slow}$), probably because of lack of micropores and large solubilization effect of bile salts.

Bioaccessibility of Phenanthrene on CNTs through the In-vitro Gastrointestinal System. The bioaccessibility of adsorbed HOCs on CNTs in the gastrointestinal tract is of key importance for toxicity. To characterize their bioaccessibility, it is critical to understand desorption processes in the gastrointestinal tract. Phenanthrene was adsorbed to CNTs in water at 25 °C to reach a relatively high equilibrium aqueous concentration (0.95 mg/L) before the desorption process. After 2 h desorption in the gastric fluid followed by 4 h desorption in the intestinal fluid, 43−69% of phenanthrene was desorbed from CNTs in the gastrointestinal system at low bile salts concentration, while it was 53−86% at high bile salts concentration (Figure 4). Clearly, high solubilization effect of bile salts was responsible for the high bioaccessibility of adsorbed phenanthrene in the gastrointestinal system at high bile salts concentration.

The total bioaccessible percent in the gastric and intestinal fluids at high bile salts concentration (6 h = 2 + 4 h) was 53%, 65%, and 86% for SWCNT, MWCNT10, and MWCNT40, respectively (Figure 4). However, in desorption kinetics experiments, only after the rapid desorption (less than 1 h), similar or higher phenanthrene could be desorbed (75%, 61%, and 88% for SWCNT, MWCNT10, and MWCNT40, respectively) in the intestinal fluid at high bile salts concentration (Table 1). Moreover, almost 100% phenanthrene adsorbed on MWCNT40 was released after 4 h desorption in the intestinal fluid at high bile salts concentration (Table 1, Figure S7) in contrast with 86% after 6 h desorption in the gastric and intestinal fluids at high bile salts concentration. Therefore, it can be concluded that pepsin was adsorbed on CNTs during the phenanthrene desorption process in the gastric fluid, and the adsorbed pepsin suppressed the desorption of phenanthrene from CNTs during the desorption process in the intestinal fluid. There are two possible reasons for the above observation: (i) after desorption in the gastric fluid (2 h), a fraction of adsorbed phenanthrene was still in the micropores for all CNTs as discussed above from the desorption kinetic results (Table 1). Pepsin is an amphiphilic biomolecule and the hydrophobic residues account for 35% of the total residues.[32] Bile salt molecules could be adsorbed on the pepsin surface upon contact[37,38] which could decrease the solubility enhancement due to the reduction of available bile salt molecules to form micelles. As a result, the release of phenanthrene from micropores was delayed. (ii) Adsorbed pepsin was dissociated and desorbed from CNTs when pH of the fluid changed from 2.0 to 7.5 in the intestinal fluid,[39] more surface sites were thus exposed and occupied by phenanthrene molecules.

The no observed adverse effect level of phenanthrene to human is about 7.5 mg/kg/day.[40] A worst case scenario was analyzed based on the saturated adsorbed phenanthrene before desorption (220, 50, and 15 mg/g for SWCNT, MWCNT10, and MWCNT40, respectively) and phenanthrene bioaccessibility in the simulated gastrointestinal fluid (Figure 4). Calculated maximum allowable oral intake amounts of SWCNT, MWCNT10, and MWCNT40 are 79, 290, and 720 mg/kg/day, respectively in the gastrointestinal fluid at low bile salts concentration while 64, 229, and 582 mg/kg/day in the gastrointestinal fluid at high bile salts concentration. These intake levels will become lower for more toxic compounds with higher hydrophobicity. The peroral toxicity of purified CNTs was studied and no toxicity was observed for rats and mice even when CNTs concentration was over 1000 mg/kg of body weight.[9,10] Thus, adsorbed HOCs could pose more risk than CNTs alone and should be considered in peroral toxicity assessments. Furthermore, CNTs should be purified for various applications (e.g., food packaging and medical applications) and CNTs used for wastewater treatment plants should be processed to remove adsorbed HOCs and other contaminants before disposal.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. The influence of pepsin/bile salts on ^{14}C-labled phenanthrene measurement (Figure S1); Adsorption of phenanthrene on CNTs on a unit surface area basis (Figure S2); Phenanthrene adsorption on CNTs in background, NaCl−HCl, and NaCl−Na_2CO_3 solutions (Figure S3); Pepsin structure and phenanthrene solubility (Figure S4); Surface tension of bile salts and phenanthrene solubility in bile salts solutions (Figure S5); Competition between phenanthrene and pepsin or bile salts (Figure S6); Desorption of phenanthrene from CNTs (Figure S7); Selected properties of CNTs (Table S1); Langmuir fitting results of phenanthrene isotherms (Table S2); Freundlich (Table S3) and Langmuir (Table S4) fitting results of pepsin and bile salts isotherms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +1 413 545 5212; fax: +1 413 545 3958; E-mail: bx@pssci.umass.edu.

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