**Adsorption and bioaccessibility of phenanthrene on carbon
nanotubes in the *in vitro* gastrointestinal system**

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ABSTRACT

Adsorption and bioaccessibility of phenanthrene on graphite and multiwalled carbon nanotubes (CNTs) were investigated in simulated gastrointestinal fluid using a passive dosing system. The saturated adsorption capacity of phenanthrene on different adsorbents follows an order of hydroxylated CNTs (H-CNTs) > carboxylated CNTs (C-CNTs) > graphitized CNTs (G-CNTs) > graphite, consistent with the order of their surface area and micropore volume. The change of phenanthrene adsorption on the adsorbents is different with the presence of pepsin (800 mg/L) and bile salts (500 mg/L and 5000 mg/L, abbreviated as BS500 and BS5000). Both solubilization of phenanthrene by pepsin and bile salts and their competition with phenanthrene for the adsorption sites play a role. In addition, the large increase of the maximum adsorption capacity in BS5000 solution indicates an enhanced dispersion of CNTs or an exfoliation of graphite by bile salts, which consequently increases the exposed surface area. The bioaccessibility increases in pepsin and BS500 solution with a growing free phenanthrene concentration. Although the bioaccessibility of phenanthrene stalls or slightly decreases in the middle range of free phenanthrene concentration in BS5000 solution, the bioaccessibility overall is much higher than that in pepsin and BS500 solution at the same phenanthrene level. It is impossible to separate the effect of competition from dispersion (or exfoliation) at this stage, but the relative contribution of solubilization to phenanthrene desorption in pepsin and BS500 solutions was quantified, which improves our understanding of the mechanisms on bioaccessibility of adsorbed pollutants on CNTs.

Keywords:

adsorption; solubilization; CNTs; passive dosing; gastrointestinal fluid

1. Introduction

Carbon nanotubes (CNTs) have been produced in a remarkably growing rate and been widely applied in wastewater treatment, food packaging, medicine delivery and other fields (De Volder et al., 2013; Heister et al., 2013; Wang et al., 2012). Consequently, human exposure risk to CNTs has increased, and oral ingestion is considered as one of the key pathways. CNTs can also enter the food chain by the uptake of plants (Khodakovskaya et al., 2009) and fish (Handy et al., 2008), and hence be potentially ingested by human. CNTs themselves may have adverse health impacts (Helland et al., 2007), but more importantly, due to the superior adsorption capacity, CNTs can adsorb a great amount of toxic contaminants like PAHs. As a representative PAH, phenanthrene can be attach on CNTs mainly through two possible ways: (1) either as the carbon sources (Lai et al., 2001) or as the byproduct (Plata et al., 2009) during the CNTs synthesis; (2) through interacting in the environmental media including air and water (Ren et al., 2011). The adsorption of phenanthrene on CNTs are influenced by the properties of CNTs such as surface area, micropore volume and functional groups (Yang and Xing, 2010). Adsorbed phenanthrene on CNTs can be further released in the gastrointestinal tract after ingestion (Wang et al., 2011). As a result, it is of great importance to understand the bioavailability of CNTs-adsorbed phenanthrene and its driving factors in the gastrointestinal fluids.

In-vitro gastrointestinal model has been extensively applied to investigate the bioaccessibility of adsorbed hydrophobic organic pollutants (HOPs) on soil (Tao et al., 2011), food (Goñi et al., 2006) and even CNTs (Wang et al., 2011). The desorption of HOPs from adsorbent is mainly driven by the solubilization of HOPs in gastrointestinal fluids and by the competition from components of gastrointestinal fluids like pepsin and bile salts with HOPs on adsorbent. Using the conventional batch/centrifugation, it is impossible to determine the relative contributions of solubilization and competition, because centrifugation cannot separate the pepsin and bile salts from the aqueous phase (Wang et al., 2011; Zhao et al., 2012). For example, in our previous study, the bioaccessibility of phenanthrene on multiwalled CNTs (MWCNTs) in *in-vitro* gastrointestinal system (simulated

gastrointestinal digestion: 2 h in gastric fluid followed by 4 h in intestinal fluid) ranged from 43% to 86%, but the exact contributions of competitive adsorption and solubility enhancement were not identified (Wang et al., 2011). However, a passive dosing method with constant freely dissolved concentrations of analytes (C_{free}) fills this gap. The mechanism of a passive dosing system is to hold a relatively stable C_{free} through equilibrium partitioning between aqueous solution and the preloaded silicone (Birch et al., 2010). Passive dosing method has been employed in the studies on bioavailability of phenanthrene simulated lung fluid (Zhao et al., 2012), bioavailability of pyrene associated with suspended sediment (Zhang et al., 2015), and competitive adsorption of surfactant (sodium cholate) with phenanthrene (Zhao et al., 2014b). In contrast to the solubilization and competition that both can enhance the bioavailability, the possibly improved dispersion of CNTs caused by the components in gastrointestinal fluids may lead to a reduction of bioavailability owing to the increasing adsorption surface area of CNTs (Zhao et al., 2015).

The objective of this study is to quantitatively separate the contribution of solubilization to the bioaccessibility of phenanthrene on different carbon materials including graphite, graphitized MWCNTs (G-CNTs), hydroxylated MWCNTs (H-CNTs) and carboxylated MWCNTs (C-CNTs) in the simulated gastrointestinal fluids using passive dosing devices. The influence of different properties of carbon materials on adsorption and the driving forces of bioaccessibility like solubilization, competition and dispersion are also discussed.

2. Materials and methods

2.1. Materials and experiment preparation

Radioactive (^{14}C labeled) phenanthrene (8.2 $\mu\text{Ci}/\mu\text{mol}$) and unlabeled phenanthrene were purchased from Sigma-Aldrich Chemical Co. The molecular weight, octanol-water partition coefficient ($\log K_{\text{ow}}$), and water solubility at 37 °C of phenanthrene are 178.2 g/mol, 4.57, 1.75 mg/L, respectively (Yang et al., 2006). Graphite and the three types of CNTs (G-CNTs, H-CNTs and C-CNTs) were purchased from

Chengdu Organic Chemistry Co., Chinese Academy of Sciences. H-CNTs and C-CNTs were produced in HCl solution at different temperatures with different oxidation levels of KMnO_4 . The characteristics (surface area, micropores volume, surface oxygen content and total oxygen content) of these adsorbents are summarized in [Table S1](#).

Both pepsin and bile salts were also obtained from Sigma-Aldrich Chemical Co. Pepsin (600-1800 units/mg protein) with a molecular weight of 35 kDa is from porcine gastric mucosa, and bile salts comprise 50% sodium deoxycholate (NaDC) and 50% sodium cholate (NaC). Human gastrointestinal compositions are simulated in terms of ionic strength and pepsin and bile salts contents (Kararli, 1995; TenHoor et al., 1991). The simulated gastric fluid contains 800 mg/L pepsin in a solution of NaCl-HCl (NaCl at 0.1 mol/L, pH at 2.0). Two intestinal fluids with either low (500 mg/L, referred to as BS500) or high (5000 mg/L, BS5000) concentrations of bile salts were prepared in a neutral buffered solution (NaCl at 0.12 mol/L, Na_2CO_3 at 0.02 mol/L, pH at 7.5), representing the fasted-state and fed-state conditions of digestion. A solution of 0.01 mol/L CaCl_2 in distilled water (pH at 7.0) was used as a background solution to compare with gastrointestinal fluid for the adsorption experiments. A concentration of 200 mg/L of NaN_3 was maintained in all solutions to prevent the phenanthrene degradation.

The passive dosing technique has been reported in detail by [Birch et al. \(2010\)](#) and has been widely used in the adsorption experiments (Zhao et al., 2012, 2014). In brief, the prepolymer and catalyst (10:1, w/w) from the poly (dimethylsiloxane) (PDMS) elastomer kit (Silastic MDX4-4210, from Dow Corning Co.) were mixed first. The mixture of silicone (600 ± 5 mg) was deployed in each 20 mL vial, which was then sealed and kept at 4 °C for 2 days to remove the bubbles in the silicone. The vials were placed at room temperature for 72 h and at 110 °C for another 48 h to cure. The silicone was cleaned for three times using methanol (HPLC grade, Fisher) after curing to fully get rid of impurities and oligomers. Each cleaning lasted for 12 h at least and performed at the same temperature as the following adsorption experiments (37 °C). The vials were rinsed by ultrapure water three times to remove the methanol and wiped with lint-free tissue.

Unlabeled phenanthrene was diluted in methanol and then mixed with ^{14}C labeled phenanthrene to obtain a series of concentrations (10-3000 mg/L). 800 μL of each phenanthrene solution, 500 μL and 1 mL of ultrapure water were added to each vial at 30 min intervals in sequence, in order to drive the phenanthrene loading into silicone. After 12 h, another 17.7 mL ultrapure water was added to each vial, and the vials were shaken at 37 °C for 3 days. The solution in the vials was replaced by 20 mL ultrapure water and shaken for 24 h repeatedly until the C_{free} in the water reaching constant. Meanwhile, the methanol can be also fully removed. The liquid scintillation counting (Beckman LS6500) was adopted to determine C_{free} from the mixture of 1 mL solution and 4 mL Ultima Gold XR cocktail (Perkin-Elmer). Thus, a batch of loaded vials with different phenanthrene amounts was obtained.

2.2. Solubilization experiments

The experiments of phenanthrene solubilization by pepsin and bile salts were conducted using the loaded vials. 20 mL of pepsin solution, BS500 or BS5000 solution were added to passive dosing vials that were pre-loaded with different concentrations of phenanthrene. The vials were shaken at 37 °C for 5 days to reach equilibrium. A mixture of 1.6 mL solution from the vial and 4.0 mL of cocktail was deployed in the liquid scintillation counter to determine the total phenanthrene concentration (C_{total}) in the solution. After discarding the remaining solution, the vials were quickly rinsed using background solution for five times and then wiped with lint-free tissue. 20 mL of background solution was added to these vials, and the phenanthrene concentration in the solution was measured after equilibrium, which was adopted as a surrogate for C_{free} . The difference between C_{total} and C_{free} , thus, is the enhanced concentration of phenanthrene ($C_{\text{solubilized}}$) by the solubilization of pepsin or bile salts.

2.3. Adsorption Experiments

All adsorption experiments were conducted using the passive dosing vials with a series of phenanthrene concentrations. Each vial was added by 0.8 mg of CNTs or 2.0 mg of graphite, followed by 20 mL of background, pepsin, BS500 or BS5000 solution. The sealed vials were shaken on a temperature-

controlled shaker (37 °C) for 5 days to reach equilibrium. The solution in each vial was then transferred to another vial, and the adsorbent on the vial wall and silicone was carefully rinsed using the transferred solution to make sure that all the adsorbent has been transferred. The used passive dosing vials were added by 20 mL of background solution, and the C_{free} was determined after equilibrium. The new vials with transferred solution were ultrasonicated (42 kHz, 100 W) for 1 h to suspend the adsorbent, and then 1 mL of the well-suspended solution was mixed 4 mL cocktail to measure the total phenanthrene concentrations (C_{total}). The measured C_{total} here is the sum of C_{free} , phenanthrene solubilized by pepsin or bile salts ($C_{\text{solubilized}}$), and the adsorbed phenanthrene on the adsorbent (C_{adsorbed}). The equilibrium adsorbed concentration of phenanthrene on the adsorbent (q_e) was calculated as:

$$q_e = (C_{\text{total}} - C_{\text{free}} - C_{\text{solubilized}}) / C_{\text{adsorbed}} \quad (1)$$

where C_{adsorbed} is the concentration of the adsorbent (graphite, G-CNTs, H-CNTs or C-CNTs).

The loss of pepsin and bile salts caused by the adsorption on adsorbents was also examined. The same amount of adsorbent (2.0 mg graphite or 0.8 mg CNTs) was added to a 20 mL vial. Then the vials both with and without adsorbents were filled with 20 mL of background, pepsin, BS500 or BS5000 solution. After shaking at 37 °C for 5 days, the vials was centrifuged, and the pepsin or bile salts concentration in the supernatant was measured on a Total Organic Carbon Analyzer (TOC-5000A, Shimadzu) using the same method of [Wang et al. \(2011\)](#).

2.4. Data analysis

All experiments were performed in triplicate. The data of phenanthrene adsorption on graphite and CNTs were fitted by the Dubinin-Ashtakhov (DA) model (Yang and Xing, 2010):

$$q_e = \frac{Q_0}{10^{(\frac{q_e}{E})^b}} \quad (2)$$

where Q_0 is the maximum adsorption capacity (mg/kg); E and b stand for the “correlating divisor” (kJ/mol) and a fitting parameter, respectively. ε is the effective adsorption potential (kJ/mol), calculated from:

$$\varepsilon = -RT \ln(C_{\text{free}} / C_s) \quad (3)$$

where R , T and C_s represent the universal gas constant (8.314×10^{-3} kJ/mol K), the absolute temperature (K), and the water solubility of phenanthrene (mg/L), respectively.

3. Results and Discussion

3.1. Solubilization of phenanthrene by pepsin and bile salts

A linear regression was employed to fit the solubilization effect of phenanthrene by pepsin and bile salts (Fig. 1). The coefficients of determination in the regression are greater than 0.99, and the partitioning coefficients (K) of phenanthrene between the gastrointestinal fluids and water are 1.1, 0.46 and 30 for pepsin, BS500 and BS5000, respectively (Fig. 1). Although both pepsin and bile salts enhanced the solubility of phenanthrene, the solubilization effects by pepsin and BS500 are relatively weak. There is a strong increase of phenanthrene concentrations in the solution of BS5000, consistent with a reported value (a saturated solubility by BS5000 is about 30 times of C_{free}) by Wang et al. (2011). The micelle-like aggregates formed by bile salts can trap the phenanthrene inside by their hydrophobic regions (Amundson et al., 2008), and the concentrations of bile salts in BS5000 solution (5000 mg/L) is far above the critical micelle concentration (CMC) of bile salts (around 1000 mg/L, Wang et al., 2011), which explains the large difference of phenanthrene solubility between in BS500 solution and in BS5000 solutions.

Figure 1. Solubilization of phenanthrene in pepsin, BS500 and BS5000 solutions, where $C_{total} = C_{free} + C_{solubilized}$. Note that the slope equals to $K+1$.

3.2. Phenanthrene adsorption on CNTs and graphite

The equilibrium adsorbed concentration of phenanthrene on graphite is much smaller than on CNTs (Fig. 2). The saturated adsorption capacity (Q_0) of phenanthrene on different adsorbents follows an order of H-CNTs > C-CNTs > G-CNTs > graphite (Table 1). Such differences are mainly caused by the different surface areas (A_{surf}) and micropore volumes (V_{micro}) across different carbon materials. As shown in Table S1, both A_{surf} and V_{micro} follow the same order of Q_0 : H-CNTs > C-CNTs > G-CNTs > graphite. After normalized by surface area, Q_0/A_{surf} is lower for C-CNTs and H-CNTs than that for G-CNTs and graphite (Table 1), suggesting that the presence of functional groups decreases the adsorption capacity of C-CNTs and H-CNTs. CNTs with functional groups are usually more hydrophilic because the hydroxylate or carboxylate groups can form H-bonds with water molecules, which consequently reduces the adsorption of organic compounds. The higher Q_0/A_{surf} for G-CNTs than H-CNTs and C-CNTs is also supported by the highest C/O ratio (Table S1), indicating the role of hydrophobicity of CNTs in phenanthrene adsorption (Yang and Xing, 2010). Due to the higher surface oxygen content ($O_{content}$ surface), the $Q_0/O_{content}$ surface of C-CNTs is the lowest in the three types of CNTs. Micropores on the external surface are also important sorption sites (Yang and Xing, 2010; Zhao et al., 2012). Q_0/V_{micro} of G-CNTs, H-CNTs and C-CNTs are similar and much lower than Q_0/V_{micro} of graphite (Table 1).

Both E and b follow a relationship of G-CNTs $\approx$ H-CNTs $\approx$ C-CNTs $>$ graphite in the background solution, indicating the overall interaction forces (adsorption affinity) between the three types of CNTs are similar but much higher than that of graphite. The adsorption affinity is mainly driven by hydrophobic interaction, hydrogen-bonding interaction and π -bonding interaction that only happen on the CNTs surface without functional groups (Yang and Xing, 2010). Therefore, the existence of functional groups has little influence on the phenanthrene adsorption on CNTs through hydrophobic and π -bonding interactions, leading to an almost constant E (Cho et al., 2008). Although the functional groups like –COOH and –OH may improve the adsorption affinity by forming covalent bonds with organic chemicals (Huang et al., 2002; Piao et al., 2008), this effect seems weak for the experiments here, probably due to the relatively low content of functional groups for the H-CNTs and C-CNTs (O_{content} surface 2.97% and 4.16%, respectively) used in this study (Table S1) and due to a lack of functional groups on phenanthrene to form covalent bonds.

Figure 2. Adsorption of phenanthrene on G-CNTs, H-CNTs, C-CNTs and graphite in the background solution

Table 1. The fitting results of isotherms of phenanthrene on carbon materials in the background, pepsin and bile salts solutions using Dubinin-Ashtakhov model. Q_0/A_{surf} , Q_0/V_{micro} and Q_0/O_{content} surface are the

209 normalized Q_0 by surface area (A_{surf}), micropores volume (V_{micro}) and surface oxygen content (O_{content}
 210 surface), respectively.

Adsorbent	Solution	Q_0 (mg/g)	b	E (KJ/mol)	Q_0/A_{surf} (mg/m ²)	Q_0/V_{micro} (mg/cm ³)	Q_0/O_{content} surface (mg/g)	$r^2\text{-adj}^*$
G-CNTs	background	16.6±2.3	2.10±0.33	17.7	0.31	28.0	6.23	0.962
	pepsin	5.3±1.1	1.41±0.20	14.0	0.10	9.02	2.00	0.980
	BS500	17.6±2.0	1.07±0.07	13.4	0.32	29.7	6.60	0.997
	BS5000	82.0±30.0	2.34±0.39	11.3	1.51	138	30.7	0.969
H-CNTs	background	23.5±0.9	2.44±0.12	17.8	0.25	26.0	7.93	0.995
	pepsin	7.0±1.1	1.94±0.24	14.4	0.07	7.76	2.36	0.978
	BS500	20.8±11.7	0.95±0.24	12.2	0.22	22.9	6.99	0.959
	BS5000	74.9±17.6	2.31±0.29	12.0	0.79	82.8	25.2	0.980
C-CNTs	background	19.1±1.2	2.08±0.17	17.8	0.30	28.7	4.58	0.989
	pepsin	3.9±0.6	1.74±0.31	16.7	0.06	5.83	0.929	0.966
	BS500	22.5±6.3	0.90±0.13	11.6	0.35	33.9	5.41	0.992
	BS5000	120.8±73.8	1.58±0.35	10.0	1.87	182	29.0	0.969
Graphite	background	4.7±2.0	0.97±0.21	13.5	0.57	92.3		0.970
	pepsin	0.7±0.1	2.05±0.45	19.0	0.09	13.9		0.941
	BS500	2.3±0.7	0.92±0.18	17.1	0.28	45.7		0.979
	BS5000	47.2±26.7	1.71±0.28	9.70	5.71	930		0.988

211 * $r^2\text{-adj}$ is the coefficient of determination adjusted by the number of data points (nd) and the number of fitting parameters (np),
 212 and it equals to $1 - (nd - 1) (1 - r^2) / (nd - np - 1)$.

213 3.3. Phenanthrene adsorption on CNTs and graphite with the presence of pepsin and bile 214 salts

215 The adsorption of pepsin or bile salts on CNTs and graphite was evaluated by comparing pepsin or
 216 bile salts concentrations with or without the adsorbent, and no significant difference was found (Fig. S1).
 217 The average changes of pepsin, BS500 and BS5000 are 2.5%, 0.086% and 0.025%, respectively, in the

solution with/without adsorbents. Hence, the influence of absorption of pepsin and bile salts on their solubilization of phenanthrene is negligible.

The saturated adsorption capacities (Q_0) in the pepsin solution reduced 68%, 70%, 80% and 85% for G-CNTs, H-CNTs, C-CNTs and graphite, respectively, compared to the background solution (Table 1). Similar to phenanthrene, pepsin can also occupy the same hydrophobic sites on CNTs or graphite (Wang et al., 2011) and form π -bonding with the adsorbents, and thus can be adsorbed on CNTs. The adsorption of pepsin or bile salts on CNTs acts as a competition effect for the adsorption of phenanthrene on CNTs since they share the same adsorption sites. Thus, the reduction of Q_0 is mainly due to the competition between pepsin and phenanthrene, since C_{free} is unchanged in the presence of pepsin (The solubilization of phenanthrene by pepsin is satisfied by the passive dosing system). Q_0 of CNTs in BS500 solution is similar to that in background solution (Table 1). There is no π -bonding between BS500 and CNTs due to the absence of double bonds or benzene rings in bile salts (a mixture of NaC and NaDC). The π -bonding fraction of phenanthrene on CNTs, therefore, cannot be replaced by bile salts. However, the competition for the hydrophobic sites between bile salts and phenanthrene on CNTs still exists. The similar Q_0 of CNTs between in BS500 solution and in background solution is possibly an offset between the competition (reduced Q_0) and the better dispersion (enhanced Q_0) of CNTs caused by bile salts (Haggenmueller et al., 2008; Wenseleers et al., 2004). Different from CNTs, Q_0 of graphite decreases in BS500 solution compared with that in background solution (Table 1). The bile salts can compete with phenanthrene for the adsorption sites on graphite, but it is difficult for bile salts to disperse graphite because of bulk particles of graphite and/or strong stacking between single graphitic sheets. Much higher Q_0 of CNTs was found in BS5000 solution than in background solution, especially Q_0 of C-CNTs (Table 1). It is likely that the strongly enhanced adsorption through better dispersion by high concentrations of bile salts far exceeds the competition loss of adsorption on the hydrophobic sites by bile salts. There is also a large increase of Q_0 of graphite in BS5000 solution from in the background solution. Since bile salts hardly affect the dispersion of graphite, one possible reason for the elevated Q_0 is that BS5000 may

exfoliate the graphite that increases the graphite surface area. E is reduced in the pepsin and bile salts solutions for all CNTs compared to that in the background solution (Table 1). A significant positive relationship was found between E and b for CNTs in the background, pepsin and BS500 solutions which is consistent with the linear intrinsic relationship reported by Yang and Xing (2010), but the relationship is biased for graphite and in BS5000 solution (Fig. S2).

As shown in Fig. 3, q_e of CNTs and graphite is lower in pepsin and BS500 solutions than in background solution. Considering that the solubilization of phenanthrene by pepsin or BS500 can be fully supplied by the passive dosing system, the difference of q_e between in background solution and in pepsin/BS500 solution is mostly contributed by the competition of adsorption sites between phenanthrene and pepsin/bile salts. A higher reduction of q_e in pepsin solution than in BS500 solution from the background solution was found (Fig. 3), indicating that the competition effect of pepsin is larger than BS500 (π -bonding) or an offset by enhanced dispersion in BS500 solution. It is interesting that in the BS5000 solution, q_e is smaller than that in the background solution at low free phenanthrene concentrations but gradually exceeds that in the background solution with increasing phenanthrene concentration (Fig. 3). The dispersion of CNTs or the exfoliation of graphite by BS5000 makes more exposed sites available on CNTs and graphite for the adsorption of phenanthrene and bile salts. However, the sites occupied by bile salts are relative stable, since the concentration of bile salts is fixed. In contrast, more phenanthrene can be adsorbed on CNTs and graphite with increasing free phenanthrene concentration. At a high C_{free} , thus, the increase of phenanthrene adsorption overwhelms the competition loss by bile salts, leading to a higher q_e than that in background solution. The thresholds of C_{free} (intersection between the adsorption isotherms of phenanthrene in background solution and in BS5000 solution) for the tested carbon materials follow an order of H-CNTs (0.025 mg/L) > C-CNTs (0.024 mg/L) > G-CNTs (0.017 mg/L) > graphite (0.0052 mg/L). The thresholds of C_{free} are significantly correlated to the surface area of each adsorbent (Fig. S3), which sheds light on the mechanism that the exceeding q_e in BS5000 solution after the threshold is likely due to the new exposed sites by enhanced

dispersion or exfoliation. It should be noted, however, more direct evidence is needed to fully explain this phenomenon.

Figure 3. Adsorption of phenanthrene on G-CNTs, H-CNTs, C-CNTs and graphite in background, pepsin and bile salts solutions.

3.4. Bioaccessibility of phenanthrene on carbon materials in simulated gastrointestinal fluids

The bioaccessibility of phenanthrene adsorbed on carbon materials (bioaccessibility = $(C_{\text{solubilized}} + C_{\text{free}}) / C_{\text{total}}$) is depicted in Fig. 4. In pepsin and BS500 solutions, bioaccessibility of phenanthrene adsorbed on CNTs and graphite increases with growing C_{free} (Fig. 4), implying the higher potential health risk with increasing adsorbed phenanthrene. Phenanthrene on graphite exhibits higher bioaccessibility than

on CNTs, due to the lower surface area, thus resulting in lower phenanthrene adsorption and relatively stronger competition by pepsin and bile salts. The force of adsorption is also stronger for CNTs than for graphite (higher E in Table 1). The bioaccessibilities for the three types of CNTs are similar in BS500 solutions, but slightly different in pepsin solutions. With increasing C_{free} , bioaccessibility of phenanthrene on H-CNTs is lower than that on G-CNTs and on C-CNTs (Fig. 4), mainly caused by the larger surface area of H-CNTs (Table S1), hence smaller desorption fraction. For a given carbon material, the bioaccessibility of phenanthrene is lower in BS500 solution than that in pepsin. It is likely that the enhanced dispersion of CNTs or exfoliation of graphite by bile salts, thus more adsorption sites available, compensates for the effects of competition and solubilization. On the other hand, without dispersion or exfoliation, the competition and solubilization are the mainly responsible for the bioaccessibility of phenanthrene in pepsin solution.

As C_{free} increases in BS5000 solution, the bioaccessibility of phenanthrene sharply increases first, then stays stable or slightly decreases, and keeps increasing moderately at the end (Fig. 4). The bioaccessibility in BS5000 solution is rather complicated because it is determined mutually by three forces: 1) the competition of bile salts with phenanthrene for the adsorption sites on CNTs or graphite; 2) the solubilization of phenanthrene by bile salts; 3) the enhanced surface area as a consequence of better dispersion of CNTs or exfoliation of graphite driven by bile salts. The first two effects can improve the bioaccessibility of phenanthrene on carbon materials while the last process would reduce it. It appears that at lower and higher C_{free} levels, the competition and solubilization outpace the effects of better dispersion or exfoliation, resulting in an increasing trend of bioaccessibility (Fig. 4). But in the middle range of C_{free} , the effects of dispersion and exfoliation seem dominant, which maintains a stalling or slight decreasing of bioaccessibility (Fig. 4). Although there are changes in the trend of bioaccessibility along a series of C_{free} , it should be noted that the bioaccessibility in BS5000 solution sharply goes up to above 60% at very low C_{free} level, much higher than that in pepsin or BS500 solutions at the same C_{free} level. In other words, the

potential risk may get even higher due to the larger fraction of phenanthrene desorbed from carbon materials at low level of C_{free} .

Figure 4. Bioaccessibility of adsorbed phenanthrene on different carbon materials in the solutions of pepsin and bile salts. The bioaccessibility is calculated from $\text{bioaccessibility} = (C_{\text{solubilized}} + C_{\text{free}}) / C_{\text{total}}$.

The relative contributions of solubilization to the desorbed phenanthrene on different materials by pepsin and BS500 were further calculated (Fig. 5). The solubilization contribution in BS5000 is not shown because there is no desorbed fraction in BS5000 solution as a result of the large dispersion effects by BS5000 (see Fig. 3, higher q_e of carbon materials in BS5000 solution than in background solution at high C_{free} level). The solubilization of pepsin and BS500 contributes more for the phenanthrene desorption from graphite than from CNTs (Fig. 5). Only with competition effect and without dispersion effect, the contributions of solubilization by pepsin increase monotonically along a growing C_{free} for all carbon materials, reaching 81% for graphite and 55% on average for CNTs at high C_{free} level. But the solubilization contribution by BS500 declines first (slightly for CNTs and largely for graphite) and increases afterward. The minimum solubilization contribution by BS500 is about 45% for graphite. It appears that the overall effects of competition and dispersion increase faster than the effect of solubilization at low C_{free} level, and then become slower at high C_{free} level. At this stage, however, it is difficult to quantitatively separate the relative contributions from competition and dispersion.

Figure 5. The contribution of solubilization to the desorbed phenanthrene amount from different carbon materials in pepsin and BS500 solutions.

4. Conclusion

The properties of different carbon materials like surface area, micropore volume and functional group content can impact their adsorption capacity of phenanthrene. With the presence of pepsin and bile salts, the adsorption of phenanthrene on different adsorbents changes due to the solubilization of phenanthrene by gastrointestinal fluids, the competition for surface adsorption sites on adsorbents and the possible enhanced surface area through dispersion or exfoliation. Using the passive dosing technique, the contribution of solubilization to phenanthrene desorption from CNTs and graphite by pepsin and BS500 are successfully quantified. Yet, the relative contributions of competition and dispersion for CNTs (or exfoliation for graphite) by bile salts cannot be determined using the current procedure. More direct evidence of dispersion and exfoliation and their contribution to phenanthrene sorption/desorption are needed in the future with a different experimental approach.

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Supplementary data

Supplementary data related to this article can be found online.

Reference

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