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Comparison of sorption kinetics of PAHs by sorptive sinks and caco-2 cell and the correlation between bioaccessibility and bioavailability of PAHs in indoor dust

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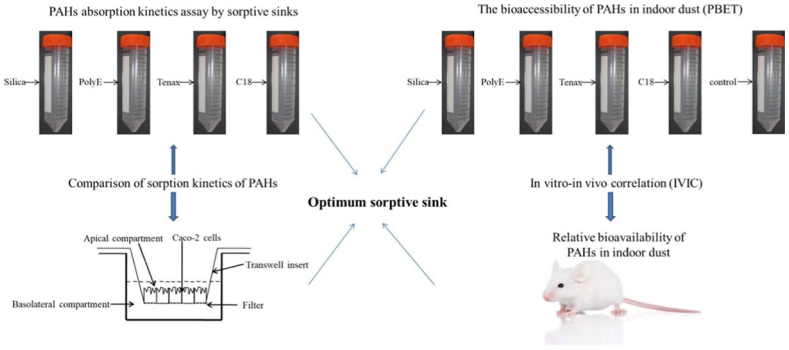
HIGHLIGHTS

- The elimination rate of PAHs by caco-2 cell was similar to silica and polyE.
- The sorptive sinks added led to 1.17–8.47-fold enhancement of bioaccessibility.
- Silica and polyE have the potential to simulate caco-2 cell.
- PBET with silica or polyE is likely to predict PAHs RBA in indoor dust.

GRAPHICAL ABSTRACT



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ABSTRACT

Sorptive sinks are extensively used in the bioaccessibility of organic contaminants, but their suitability for simulating the intestinal cell is seldom reported. In the present study, the sorption efficiency of PAHs by sorptive sinks including silica, poly(ethylene-*co*-vinyl acetate) (polyE), tenax, and C18 were compared with that by caco-2 cells. The elimination rate constants of phenanthrene, fluoranthene, pyrene, benzo(a)pyrene by caco-2 cell were 0.0417 ± 0.006 min^{-1}, 0.0411 ± 0.0074 min^{-1}, 0.0362 ± 0.006 min^{-1}, and 0.0526 ± 0.0037 min^{-1}, respectively, which were more closely to that of silica and polyE compared to other materials. This indicated that these materials might be the preferable sorptive sinks to simulate absorption of PAHs by intestinal cells. The bioaccessibility of phenanthrene, fluoranthene, pyrene, benzo(a)pyrene in indoor dust ranged from 15.5–43.5%, 9.10–38.8%, 10.0–37.9%, and 6.00–21.9%, respectively, based on physiologically based extraction test (PBET) and the sorptive sinks added in the intestinal solution led to 1.17 to 8.47-fold enhancement of bioaccessibility. The correlation of *in vivo* PAHs relative bioavailability (RBA) and *in vitro* digestion bioaccessibility with or without

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PAHs
Indoor dust

the sorptive sinks of indoor dust were measured, and the results indicated that silica and polyE were more likely to predict PAHs RBA of indoor dust, which was consistent with the results of sorption kinetics assay. The present results indicate that silica and polyE have the potential to simulate caco-2 cell and the inclusion of these materials in the PBET is likely to predict PAHs RBA in indoor dust.

Capsule: Silica and polyE were more likely to simulate absorption of PAHs by intestinal cells, and to predict PAHs RBA of indoor dust.

1. Introduction

Indoor dust serves as a sink and repository for semi-volatile organic compounds including polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants and polychlorinated biphenyls (Kang et al., 2011; Liagkouridis et al., 2014). Non-dietary ingestion of indoor dust could occur especially for children due to their frequent hand-to-mouth activities (Maertens et al., 2004). Indoor dust could significantly contribute to the bioaccumulation of organic pollutants such as PBDEs and PAHs in human body (Ali et al., 2016; Canbaz et al., 2016; Venier et al., 2016). In addition, children exposure to PAHs *via* indoor dust has already raised great concerns. A substantial number of studies have reported PAHs contamination profiles in indoor environment and risk assessments have been performed based on the assumption that all PAHs in the settled indoor dust were solubilized in the gastrointestinal juice and absorbed by the intestinal cells (Lohmann et al., 2000; Maertens et al., 2008; Kang et al., 2015; Ali, et al., 2016). However, to achieve a sound evaluation of risks associated with PAHs in indoor dust, the oral bioavailability of PAHs from the dust samples should thus be considered.

Generally, the oral bioavailability of contaminants in dust could be categorized into four steps: (1) soil ingestion, (2) release from the solid matrix into the digestive fluid, (3) intestinal absorption, and (4) liver metabolism (Oomen et al., 2001). Several studies investigated the absolute bioavailability of PAHs in contaminated soils using different animal models (Duan et al., 2016). James et al. found that the PAHs bioavailability in 9 soil samples ranged from 0.01 to 29%, based on swine blood serum as the endpoints (James et al., 2011). Pu et al. observed that the phenanthrene (Phe) bioavailability in spiked soil ranged from 15 to 49%, based on Phe quantification in blood using mice model (Pu et al., 2004). Although *in vivo* studies are more likely to simulate the "real life situation" of contaminants in human body, the associated high expense, heavy labor and ethical dilemmas limit its wide application. Therefore, several *in vitro* digestion methods including physiologically based extraction test (PBET), *in vitro* gastrointestinal method (IVG), simulator of the human intestinal microbial ecosystem (SHIME), fed organic estimation human simulation test (FOREhST) and Dutch National Institute for Public Health and the Environment (RIVM) method have been established for bioaccessibility measurement of contaminants (such as PAHs) in soils or indoor dust to estimate their bioavailability (Ruby et al., 1996; Tang et al., 2006; Lu et al., 2010; James et al., 2011; Kang et al., 2011). The most studied *in vitro* digestion model for measurement of PAHs bioaccessibility is PBET. In addition, a unified format related to PBET and IVG methods for bioaccessibility test of organic pollutants was proposed by Collins et al. (2015) based on a comprehensive review.

The *in vitro* digestion methods mentioned above are only able to simulate the mobilization of contaminants from environmental matrices into the digestion juice, and the process lacks the simulation of intestinal absorption. That would result in lower estimation of bioavailability of contaminants. To solve this problem, some studies employed the absorptive sinks such as silicone rod (Gouliarmou and Mayer, 2012; Gouliarmou et al., 2013; Zhang et al., 2015; Juhasz et al., 2016) and C18 membrane (James et al., 2011) to simulate the passive molecular diffusion of contaminants across the small intestines in assisting bioaccessible extraction of PAHs from soil or fuel soot, which were more comparable to *in vivo* studies. C18 membrane or silicon rods are not amenable to back-extraction, and require large surface areas (~2 m) to ensure a high capacity of adsorption. Therefore, tenax beads (TA) and poly(ethylene-*co*-vinyl acetate) have been selected as effective materials to assess the bioaccessibility of contaminants such as PAHs, DDTr and PBDEs in soil or house dust due to their greater adsorption capacity and easy back extraction (Vasiluk et al., 2007; Fang and Stapleton, 2014; Li et al., 2016; Cipullo et al., 2018). These studies made a breakthrough in estimation of bioavailability of contaminants. However, the sorption abilities and kinetic characteristics of these sorption materials have not been validated, and to what extent do their capacities be comparable to intestinal cell is not certain.

Caco-2 cells, an enterocyte cell line dervied from a human colon adenocarcinoma, have been extensively used as a model for evaluation of drug transport across the intestinal epithelial cell, and which may be able to assess POPs transport in intestinal phase (Cui et al., 2016). However, the caco-2 cell mode was expensive and laborious. Therefore, comparison of sorptive materials and caco-2 cell could be made based on the sorption efficiency of PAHs, in order to evaluate the suitability of those "sorptive sinks" to substitute the caco-2 model. With the above background, the objectives of the present study were to (1) test the uptake efficiency of PAHs by caco-2 cell model; (2) compare the sorption efficiency of PAHs by sorptive sinks with that of caco-2 cells; (3) examine the bioaccessibility of PAHs in indoor dust samples with or without the assistance of sorptive sinks; (4) investigate the relative bioaccessibility (RBA) of PAHs in indoor dust samples using mice model; and (5) correlate RBA of PAHs to bioaccessibility determined by *in vitro* digestion assays with or without the sorptive sinks.

2. Materials and methods

2.1. Uptake of PAHs by Caco-2 cell

Caco-2 cell line (American Type Culture Collection, Rockville, USA) was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA), supplemented with 20% (v/v) fetal bovine serum (Gibco, USA), 1% sodium pyruvate, 1% (v/v) non-essential amino-acid (Gibco, USA), and 1% antibiotics (Gibco, USA), and incubated at 37 °C, 5% CO_2 in air atmosphere (Pan et al., 2016). After the culture growth between 80% and 100% of confluency, cells were collected by treating with 0.25% trypsin-EDTA and then seeded onto Corning transwell inserts (6 well, 24 mm diameter, 0.4 μm pore size) for 2–3 weeks. The transwell system consisted of an apical and a basolateral side, which represented the intestinal lumen and the blood and lymph drainage, respectively. When the cells in inserts grew at 100% of confluency, PAHs in DMEM (100 ng mL^{-1}, 1.5 mL) were added at the apical side, and 2.6 mL DMEM to the basolateral compartment to perform the PAHs uptake assay.

Four PAHs including phenanthrene (Phe), fluoranthene (Flu), pyrene (Pyr), and benzo(a)pyrene (B(a)P) were selected to carry out the transport assay. The cell monolayers were incubated with PAHs spiked apical medium for various periods of time up to 12 h (0 h, 1 h, 3 h, 6 h, and 12 h; $n = 3$ per time interval). A 200 μL aliquot of the apical medium was collected to determine lactate dehydrogenase (LDH) leakage of the cells. Afterwards, the basolateral medium and the apical medium were sampled. The cell layer was washed with methanol three times, and the washing solution was combined for PAHs determination.

Cells were collected after treating with trypsin-EDTA. The filter was washed once with 2 mL phosphate buffered saline (PBS) and once with 2 mL hexane, and the washes were added in the cell samples. Mass balance was performed by analyzing the PAHs levels in apical, basolateral, washing and cell samples. The collected apical, basolateral and washing were spiked with known amounts of surrogates (phenanthrene-d_{10} and chysene-d_{12}), and extracted with 5 mL hexane three times. The cell samples were also spiked with known amounts of surrogates and disrupted by freeze-thaw cycle method. The samples were then saponified with KOH solution for 1 h. The saponification solution was extracted with 5 mL hexane three times. All of the extracts were concentrated to 2 ml and purified by Florisil cleanup method (Pan et al., 2016). The eluent was then concentrated to 500 mL. Internal standards (ancenaphthene-d_{10} and perylene-d_{12}) were added in the concentrates (200 ng mL^{-1}), and stored at −20 °C until GC/MS analyses.

The absorption efficiency of PAHs by caco-2 could be determined as the following equation (Artursson and Karlsson, 1991):

$$P_{app} = \frac{dQ}{dt \times A \times C} \quad (1)$$

where P_{app} is the apparent permeability coefficients of PAHs; dQ/dt represents the rate of presence of PAHs in the basolateral compartment; A is the filter surface area (cm^2); C is the initial PAHs concentration in the apical medium.

2.2. Sorption kinetics of PAHs by sorptive sinks

Sorptive sinks including tenax beads (TA) (60/80mesh, CNW technology, Germany), poly(ethylene-*co*-vinyl acetate) (>200 μm, SIGMA-ALDRICH, USA), LC-C18 (40–63 μm, CNW technology, Germany) and silica bulk (60–200 μm, CNW technology, Germany) were employed in the present study. C18 membrane and silicone rod were commonly used in previous studies (James et al., 2011; Juhasz et al., 2016), but these materials possessed a large surface area (>2 m), which strongly affected the extraction volume. In order to compare the sorption capacities among all the sorptive sinks, LC-C18 and silica powder were selected.

PAHs sorption assay was performed in DMEM medium for better comparing the ability of absorbing PAHs with caco-2 cell. Briefly, duplicate aliquots of the DMEM solution (50 mL) were preheated at 37 °C and spiked with PAHs to achieve a final concentration of 100 μg L^{-1} for each compound. Sorptive sinks (0.2 g) were added to PAHs-spiked DMEM medium and incubated at 37 °C, 150 rpm for 30, 60, 90, and 180 min, 360 min, and 720 min ($n = 3$ per time interval). The PAH fraction remaining in the DMEM solution at extraction time t ($F_{solution}(t)$), was plotted against time. PAHs extraction was treated as described above. A one phase elimination model with a plateau was fitted to data (Gouliarmou, et al., 2013):

$$F_{solution(t)} = (1 - F_{solution(eq)}).C^{-k_1.t} + F_{solution(eq)} \quad (2)$$

where $F_{solution(t)}$ and $F_{solution(eq)}$ represent the PAHs fraction left in solution at equilibrium and t, respectively, and k_1 is the rate constant that characterizes the sorption kinetics into the sorptive sinks (Gouliarmou et al., 2013). Data were fitted by least-squares using SigmaPlot 10.0. The sorption efficiency of sorptive sinks was determined using the equation: $F_{sink} = 1 - F_{solution(eq)}$.

In order to compare the sorption ability of sinks with that of caco-2 cells, the PAHs in apical medium in the cell assay could be considered as the PAHs in the solution in the sink assay. Therefore, the k_1 that characterizes the sorption kinetics into the cells could be obtained according to the following equation:

$$F_{apical(t)} = (1 - F_{apcial(eq)}).C^{-k_1.t} + F_{apical(eq)} \quad (3)$$

where $F_{apical(t)}$ and $F_{apical(eq)}$ represent the PAHs fraction left in apical medium at equilibrium and t, respectively.

2.3. Indoor dust sample preparation and treatment

Eight settled house dust samples were collected from floor surface using vacuum cleaners in Guangzhou, south China. All household dust samples were packed with aluminum foil and filtered through a stainless-steel sieve (<100 μm) to remove large particles and debris. The organic matter (OM) contents of the dust samples were measured as described previously (Pan et al., 2016).

For PAHs analysis, the sieved dust samples were spiked with known amounts of surrogate standard (phenanthrene-d_{10} and chysene-d_{12} (Accustandard Inc., USA), with a final concentration of 100 ng g^{-1} in dust) and allowed to equilibrate for 4 h at room temperature (25 °C). The spiked samples (0.2–0.3 g) were extracted by 80 mL acetone/n-hexane (1:1, v/v) for 16 h according to the Soxhlet's procedure. Afterwards, the extracts were rotary-evaporated to approximately 2 mL, cleaned up using a florisil column, and followed by 80 mL n-hexane of elution (U.S.EPA., 1996). After the eluent was rotary-evaporated to 500 μL, internal standards (ancenaphthene-d_{10} and perylene-d_{12}) were added up to the final concentration of 200 ng mL^{-1}, and stored at −20 °C until analyses.

2.4. In vitro digestion model

An *in vitro* digestive model similar to PBET method, mimicking the gastric and small intestinal digestion processes, was used to assess the bioaccessibility of PAHs (Collins et al., 2015; Pan et al., 2016). Briefly, gastric juice contained 10.0 g/L of pepsin in 0.2 M KCl solution, of which pH was adjusted to 1.5 with HCl. Indoor dust (0.2 g) was added into 40 mL of gastric fluids and shaken at 150 rpm under 37 °C for 1 h. Each indoor dust was treated in triplicate. After centrifugation of the mixture at 7000×*g* for 10 min, the supernatant was filtered using a 0.45 mm glass fiber filter. The pellet was then resuspended by 40 ml gastric solution. The gastric solution was modified to small intestinal solution by adjusting the pH to 7.0 with $NaHCO_3$ solution, followed by adding 0.2 g of porcine bile extract (Sigma-Aldrich, USA) and 0.04 g of porcine pancreatin (Sigma-Aldrich, USA). Afterwards, tenax (0.25 g), poly(ethylene-*co*-vinyl acetate) (0.25 g), silica beads (0.25 g), and LC-C18 (in a nitrocellulose package with 5 μm pore size, 0.25 g) were individually added in the intestinal solution to serve as sorption sinks. Meanwhile, parallel treatments without sorption sinks were performed for comparison. The intestinal fluids were shaken for 4 h at 37 °C, and the supernatant was collected as described above. For the treatment without sorptive sinks, PAHs in the supernatants were extracted by liquid-liquid extraction. Briefly, known amounts of surrogate standards (phenanthrene-d_{10} and chysene-d_{12}) were spiked in 20 mL supernatant (gastric or intestinal), and extracted with 20 mL n-hexane/acetone (3:1, v/v) three times in a 250 mL separatory funnel. The pooled extracts were concentrated to 2 mL and saponified by KOH solution. The saponification solution was liquid-liquid extracted by 15 mL hexane two times. The extracts were evaporated to 2 ml, purified by Florisil column and eluted by hexane. The eluent was concentrated to 500 μL, followed by addition of the internal standards (ancenaphthene-d_{10} and perylene-d_{12}) for GC/MS analysis. For treatment with sorptive sinks, the sorptive sinks (tenax, polyE, and silica) were recovered as a previous study (Li et al., 2015) or the packages (C18) were removed from the supernatants. Afterwards, the sorptive sinks were extracted by 15 ml hexane three times after taking from the package. The pooled extracts were treated as described above but without saponification procedure. PAHs bioaccessibility was calculated by dividing PAHs extracted in gastric and intestinal phase by total dust PAHs concentration (Eq. (4)) (Pan, et al., 2016):

$$\text{PAHs bioaccessibility}\,(\%) = \frac{\text{extractable PAHs}}{\text{total PAHs}} \times 100 \quad (4)$$

where extractable PAHs = PAHs (μg) detected in gastrointestinal fluids (for the treatment without sorptive sinks) or = adding the PAHs (μg)

detected in gastrointestinal fluids and PAHs detected in the sorptive sinks (for the treatment with sorptive sinks), and total PAHs = PAHs (μg) present in dust.

2.5. In vivo mouse model

In vivo PAHs RBA studies were carried out using Balb/c mice, with body weights (BWs) ranged from 21 to 26 g. A single dose (0.4 mL) of a PAHs-spiked corn oil solution was administered to mice *via* oral gavage ($n = 3$), resulting in the dose levels of 50 μg kg^{-1} bw. Indoor dust samples (0.1 g dust sample in 0.4 mL of Milli-Qwater, $n = 8$ samples in triplicate) were also administered as a single dose *via* gavage. Mice were sacrificed by cervical dislocation at 0, 4, 8, 12, 24, and 48 h (n = 3 per time interval) and blood was collected for PAHs determination. Known amounts of surrogates (phenanthrene-d_{10} and chysene-d_{12}, 20 ng) were spiked into the blood samples, and the mixture was saponified with 5 mL methanol/KOH (8:2, v:v) at 60 °C for 1 h. Afterwards, the saponification solution was extracted with 10 mL hexane three times. The pooled extracts were concentrated to 2 mL, cleaned up using florisil column, and eluted with 80 mL n-hexane. The eluant was concentrated to 200 μL. Internal standards (ancenaphthene-d_{10} and perylene-d_{12}) were added up to 200 ng mL^{-1}, and the mixture was stored at −20 °C until GC/MS analyses.

Pharmacokinetic analysis was employed to assess PAHs RBA. The area under the blood PAHs concentration time curve (AUC) followed zero correction and dose normalization was used to calculate PAHs RBA (Eq. (5)) (Pan, et al., 2016; Ruby et al., 2016).

$$\text{PAHs}\ (\%) = \left(\frac{AUC_{[dust]}}{AUC_{[solution]}} \times \frac{D_{[solution]}}{D_{[dust]}}\right) \times 100 \quad (5)$$

where $AUC_{[dust]}$ and $AUC_{[solution]}$ represent area under the blood PAHs concentration time curve from indoor dust and PAHs-spiked corn oil administration, respectively; $D_{[dust]}$ and $D_{[solution]}$ equal to dose of PAHs administered dust and PAHs-spiked corn oil, respectively (μg kg^{-1} BW).

2.6. GC/MS analysis and QA/QC

The PAHs analysis was performed on Agilent 7890 N network GC system (30 m × 0.25 mm × 0.25 μm) equipped with 5975C inert mass selective detector. Four PAHs: were investigated in the present study. Procedural blanks, the indoor dust standard reference material (SRM2585) and sample replicates were run together with each batch of 8 samples. The variation coefficient of PAHs concentration between replicate samples was <15%. The recoveries of individual PAH in SRM2585 ranged from 72.6% for Pyr to 122% for B(a)P. The recoveries of surrogate standards (phenanthrene-d_{10} and chysene-d_{12}) in indoor dust samples, samples from sorption sinks assay, samples from caco-2 cell uptake assay, digestion juice samples, and blood samples ranged from 78.2% ± 8.28% to 118 ± 19.6%, 73.4% ± 6.62% to 94.1 ± 9.15%, 71.5% ± 7.47% to 107 ± 13.5%, 70.8% ± 6.95% to 90.3 ± 10.5%, respectively. The limit of detection (LOD) was defined as three times the standard deviation of blank readings. The LODs of Phe, Flu, Pyr, and B(a)P were 0.25 ng ml^{-1}, 0.25 ng ml^{-1}, 0.25 ng ml^{-1} and 0.5 ng ml^{-1}, respectively, in the present study.

2.7. Statistical analysis

All statistical analyses were performed using SPSS 20.0 and SigmaPlot 10.0 software. Q-Q plot and Shapiro–Wilk test was used to check the normality of the data. Linear regression analysis was employed to investigate the relationships between the *in vivo* PAHs RBA and *in vitro* data, and the organic matter and PAHs RBA of indoor dust samples. The probability value of $p < 0.05$ was set as the level for statistical significance.

3. Results and discussion

3.1. PAHs uptake by Caco-2 cell

In order to investigate the effective availability of the PAHs for cells, adsorption of PAHs to transwell insert walls was measured. About 1 to 3.5% of the total PAHs added were recovered from the apical walls cleaning after 12 h. It suggested that PAHs were effectively dissolved in the DMEM medium and available for cell absorption. Fig. S1 (supporting information) shows that all the tested PAHs were able to cross the intestinal barrier, and PAHs can be detected in the basal medium after 60 min. Mass balance assay showed the recoveries of the PAHs were larger than 92.4% at each time interval for Phe, Flu and Pyr (recovery equals to the ratio of total amount of PAHs at each time interval to the spiked amount of PAHs in apical medium, Fig. S1). This indicated that the transwell system associated with caco-2 cell line was efficient for assessing PAHs (Phe, Flu and Pyr) uptake and those three PAHs were rarely metabolized by caco-2 cells (<10%, Fig.S1). However, it can be seen from Fig. S1 that the recoveries of B(a)p were extremely low, ranging from 8.20% to 10.7%. This was possibly due to the factor that almost 90% of B(a)p was metabolized by caco-2 cells, and the results were in line with that found in a previous study (Buesen et al., 2002).

The apparent permeability coefficients (P_{app}) of Phe, Flu and Pyr were 1.58 × 10^{-6} cm s^{-1}, 1.37 × 10^{-6} cm s^{-1}, and 1.52 × 10^{-6} cm s^{-1}, respectively, which indicated the high absorption rate (>1 × 10^{-6} cm s^{-1}) of those compounds by Caco-2 cell (Artursson and Karlsson, 1991). The P_{app} of B(a)p was not available for calculation due to its high metabolization. According to the P_{app} and physicochemical properties of those compounds, it can be found that phenanthrene, the least lipophilic (log Kow = 4.6), showed the relative high absorption efficiency, whereas the Flu and Pyr with higher lipophilicity showed lower uptake rates. Similar phenomenon was observed in the study conducted by Cavret et al. (2003).

On the other hand, if PAHs on cell membrane (washing part of PAHs), in the cell, and in the basolateral compartment were considered as the bioavailable parts, the elimination kinetics of PAHs in the apical medium could be explained by the Eq. (3). Accordingly, the elimination rate (k_1) of Phe, Flu, and Pyr was 0.0417 ± 0.006 min^{-1}, 0.0411 ± 0.0074 min^{-1}, and 0.0362 ± 0.006 min^{-1}, respectively (Fig. 1). For B(a)p, if the diffusion of metabolites of B(a)p from cell to apical medium was not considered, the elimination rate (k_1) of B(a)p could be determined as 0.0526 ± 0.0037 min^{-1}, which was a higher estimation of rate constant. Normally, the elimination rates of PAHs were decreased according to their corresponding water solubility (Table S1).

3.2. Sorption kinetics of PAHs by sorptive sinks

Fig. S2 shows that different sorptive sinks possessed different elimination kinetics. For Phe, Flu and Pyr, C18 material presented the highest elimination rate, and the absorption attained a plateau at 30 min with the removing rate of larger than 95% (Fig. S2A, B, C). Poly(ethylene-*co*-vinyl acetate) (polyE) and silica demonstrated a similar pattern of removing profiles, and they reached equilibrium at 180 min only with 40–50% of elimination rate for Phe and 80–90% for Flu and Pyr (Fig. S2A), which was lower when compared with the study of Gouliarmou and Mayer (2012) using silicone rod, possibly due to different sorption solution employed. Tenax showed a relatively slow removing rate and reached a plateau at 360 min with the elimination rate of larger than 95% (Fig. S2A). For B(a)p, all of the sorptive sinks demonstrated strong sorption abilities, and all attained the equilibrium in 30 min with the removing rate of larger than 95% (Fig. S2D), which was similar to that found in other studies (Gouliarmou and Mayer, 2012; Gouliarmou, et al., 2013; Li, et al., 2015; Zhang, et al., 2015).

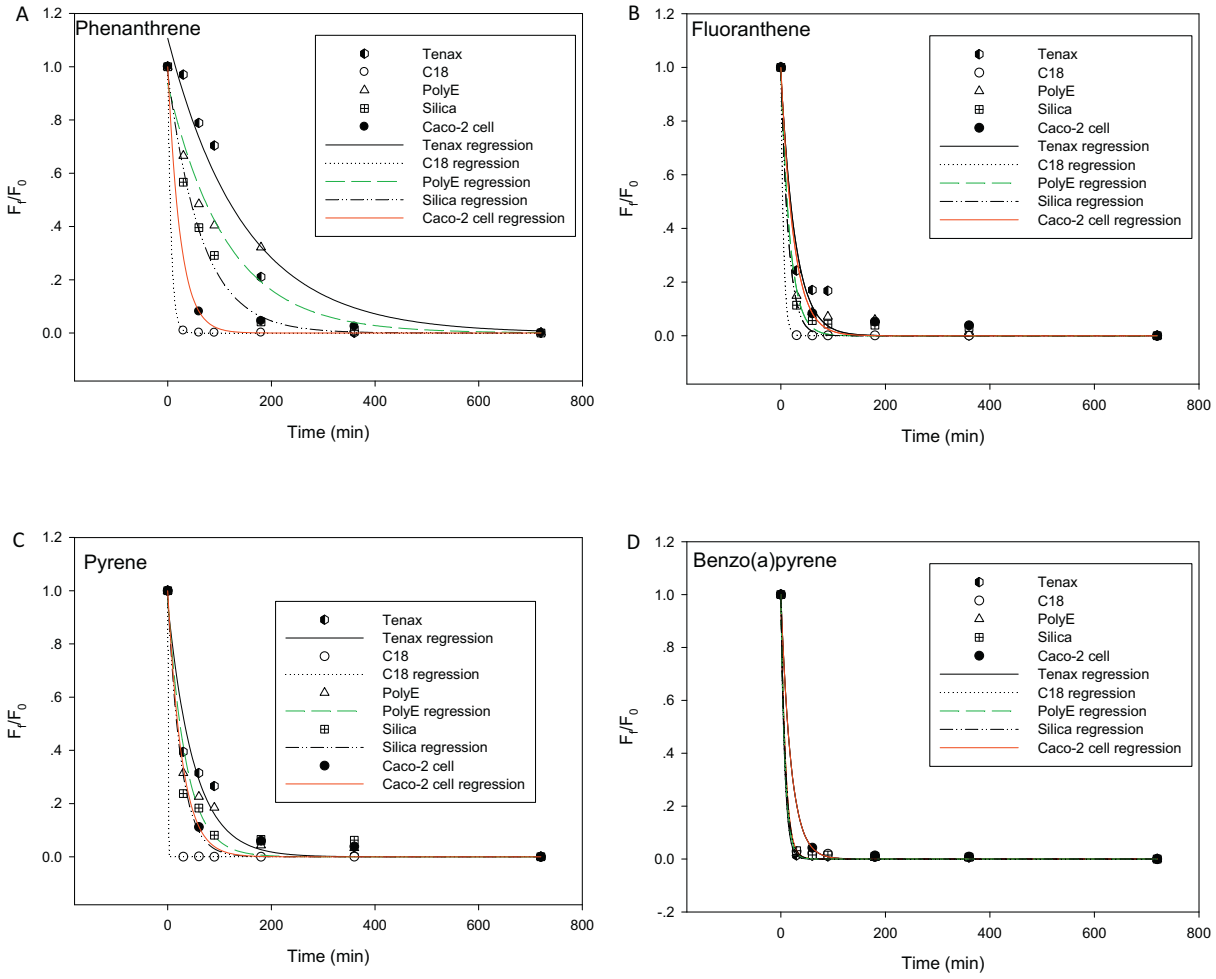

Fig. 1. The comparison of sorption kinetics of PAHs by sorptive sinks and caco-2 cells. The curves of sportive sinks were fitted by Eq. (2), and that of caco-2 cell were fitted by Eq. (3). A: Phenanthrene; B: Fluoranthene; C: Pyrene; D: Benzo(a)pyrene.

The elimination curve of those PAHs by sorptive sinks was best fitted by Eq. (2) (Fig. 1), and the elimination rate constants of Phe by polyE, silica, tenax and C18 were determined as 0.0088 ± 0.0015 min^{-1}, 0.0154 ± 0.001 min^{-1}, 0.0068 ± 0.0013 min^{-1}, and 0.1555 ± 0.007 min^{-1}, respectively. The elimination rate constant of caco-2 cell was 0.0417 ± 0.006 min^{-1}, which was close to that of silica and C18 (Fig. 1A). This may indicate that silica and C18 would be more likely to mimic the absorption kinetics of Phe by caco-2 cell. For Flu, the elimination rate constants of polyE, silica, tenax and C18 were determined as 0.0579 ± 0.0088 min^{-1}, 0.069 ± 0.009 min^{-1}, 0.0366 ± 0.0067 min^{-1}, and 0.203 ± 0.0161 min^{-1}, respectively. The elimination rate constant of Flu by caco-2 cell (0.0411 ± 0.0074 min^{-1}) was close to that of tenax, polyE, and silica (Fig. 1B). For Pyr, the elimination rate constants of polyE, silica, tenax and C18 were determined as 0.0389 ± 0.0064 min^{-1}, 0.0282 ± 0.0045 min^{-1}, 0.0198 ± 0.0033 min^{-1}, and 7.0503 ± 1 min^{-1}, respectively. The elimination rate constant of caco-2 cell (0.0362 ± 0.006 min^{-1}, Pyr) was close to that of polyE and silica (Fig. 1C). For B(a)p, all of the sorptive sinks showed similar elimination rate constants, and they were determined as 0.1253 ± 0.0009 min^{-1} (polyE), 0.1139 ± 0.0111 min^{-1} (silica), 0.1415 ± 0.0196 min^{-1} (tenax), and 0.1166 ± 0.0151 min^{-1} (C18), respectively. The elimination rate constant of caco-2 cell (0.0526 ± 0.0037 min^{-1}, B(a)p) was close to that of silica and C18 (Fig. 1BD). The results of comparison of elimination rate constants indicated that different sorptive sinks have its own advantages to simulate caco-2 cells in absorbing different PAHs, however, polyE and silica might be the preferable sorptive sinks when four PAHs of sorption kinetics were all taken into consideration.

3.3. Inclusion of four sorptive sinks for bioaccessibility measurement in indoor dust

To confirm previous results of sorption assay, the inclusion of four sorptive sinks for bioaccessibility measurement and their correlation with the *in vivo* PAHs bioavailability were investigated.

Fig. 2 shows PAHs bioaccessibility values for indoor dust determined using PBET and PBET with sorptive sinks. For PBET, Phe, Flu, Pyr and B(a)p bioaccessibility in the indoor dust, ranged from 15.5–43.5%, 9.10–38.8%, 10.0–37.9%, and 6.00–21.9%, respectively, which was higher than that found in the soil samples (below the detection limit to 13.4%) (Zhang et al., 2017) and in indoor dust samples (Kang et al., 2011), and was comparable to the that found in the study of Cave et al. (13–59%) (Cave et al., 2010).

The addition of sorptive sinks significantly increased the release of PAHs into the intestinal juice (Fig. 2). For Phe, the silica, polyE, tenax and C18 enhanced the desorption of compound into the intestinal juice by 1.53–2.68 (mean: 1.93), 1.37–2.38 (1.94), 1.56–2.53 (2.11), and 1.80–3.56 (2.78) folds, respectively (Fig. 2A). For Flu, silica, polyE, tenax and C18 enhanced the desorption of compound into the intestinal juice by 1.31–2.71 (1.92), 1.17–3.03 (1.86), 1.19–3.39 (2.02), and 1.92–7.29 (3.29) folds, respectively (Fig. 2B). For Pyr, silica, polyE, tenax and C18 enhanced the desorption of compounds into the intestinal juice by 1.27–3.33 (1.92), 1.42–3.59 (2.26), 1.64–4.54 (3.04), and 1.89–6.26 (3.95) folds, respectively (Fig. 2C). For B(a)p, silica, polyE, tenax and C18 enhanced the desorption of compounds into the intestinal juice by 2.73–5.17 (3.70),

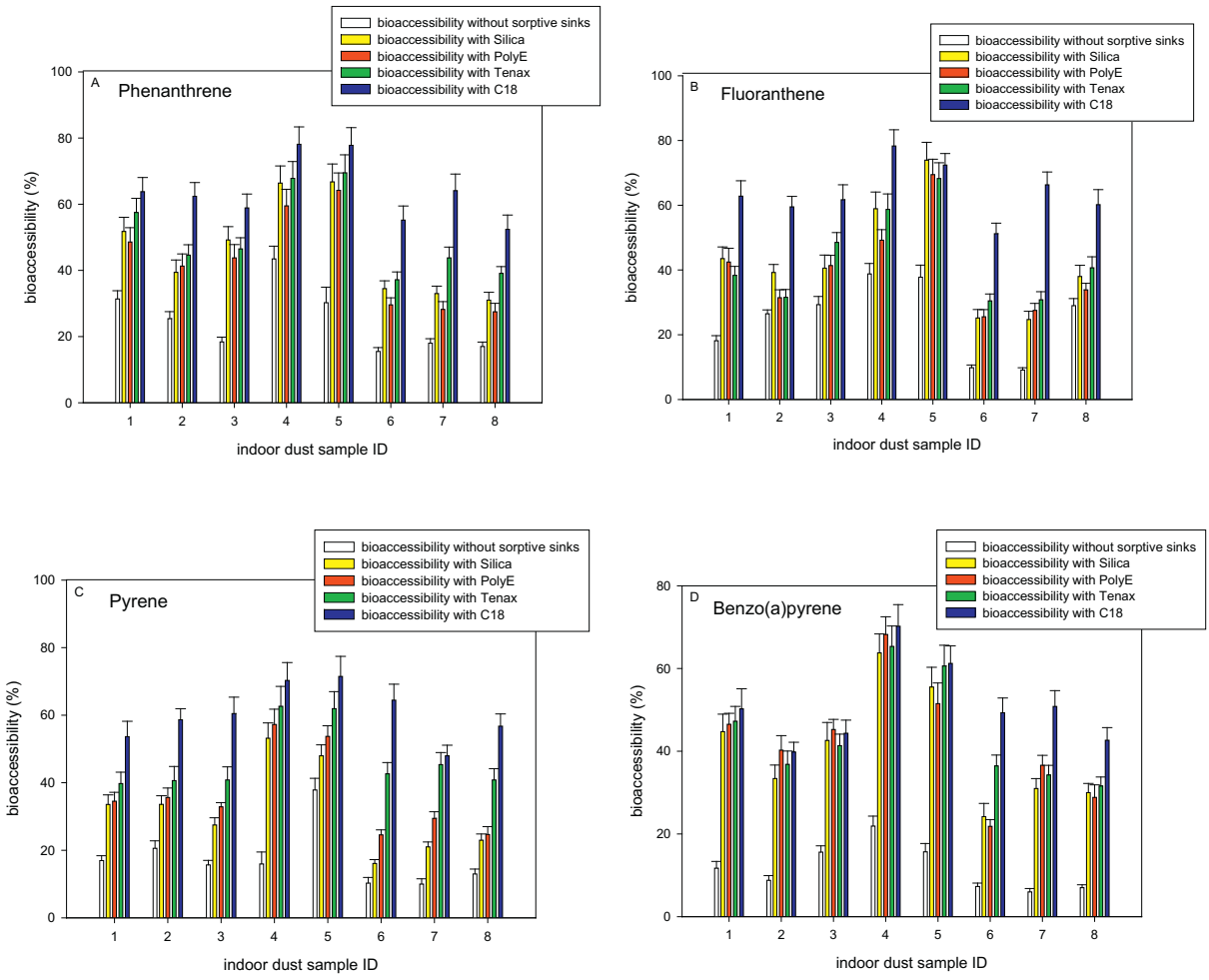

Fig. 2. Bioaccessibility of indoor dust samples with or without sorptive sinks. A: Phenanthrene; B: Fluoranthene; C: Pyrene; D: Benzo(a)pyrene.

2.90–6.10 (3.88), 2.65–5.71 (4.12), and 2.84–8.47 (5.01) folds, respectively (Fig. 2D).

It could be observed that C18 material possessed the greatest ability of facilitating the release of PAHs into intestinal solution, followed by tenax material, which is consistent with the previous sorption kinetic assay. Li et al. employed tenax to facilitate the release of PAHs into PBET intestinal juice, with 4.4-fold higher PAH bioaccessibility being found with the inclusion of tenax (Li, et al., 2015). Gouliarmou et al. used silicone rod to assist the extraction of PAHs from wood soot into intestinal juice, resulted in 2.9–24.5-fold higher PAH bioaccessibility (Gouliarmou and Mayer, 2012). James et al. added C18 membrane in the assay of Relative Bioaccessibility Leaching Procedure (RBALP), led to4.79-fold higher B(a)p bioaccessibility of contaminated soil (James et al., 2011). It should be noted that the most aggressive assisting-extraction material might not represent the optimum approach(James, et al., 2011), and the material designed to most closely simulate the *in vivo* condition needed to be established. Therefore, the comparison of different sorptive sinks and the feasibility of those sinks as a surrogate measure of PAHs RBA should be investigated.

3.4. In vivo relative bioavailability of PAHs in indoor dust

Blood concentrations of parent PAHs (usually B(a)p) can be used to determine the AUC for evaluating the RBA (Duan et al., 2014; Ruby et al., 2016). A single dose of PAHs in the corn oil was used to normalize PAHs absolute bioavailability to obtain PAHs RBA in indoor dust. Fig. S3 showed that the uptake profile of PAHs possessed three phases including absorption, distribution, and elimination (Pan et al., 2016), which could be fitted to a two-compartment model. The peak blood concentrations of Phe, Flu and Pyr appeared at 8–12 h for most of indoor dust samples and B(a)p appeared at 4–8 h, which was later than that observed in soil samples with peak at 1 h (Kadry et al., 1995; Duan et al., 2014; Duan et al., 2016). This is possibly due to the different environmental matrices used, which subsequently influenced the absorption, metabolism, and excretion rates (Ruby et al., 2016). The RBA of Phe, Flu, Pyr and B(a)p ranged from 44.7 to 90.8%, 18.9 to 71.7%, 15.0 to 56.3%, and 31.5 to 75.2%, which were obtained by the ratio of AUC of PAHs in indoor dust to AUC of PAHs in corn oil.

Studies reported the PAHs RBA in indoor dust are rare. However, PAHs RBA obtained in the present study were higher than the values reported for B(a)P in soil samples (24.2–46.1%, rat model)(Duan et al., 2016), but lower than that reported for Phe in soil samples (94–138%, rat model) (Pu et al., 2004). The difference in PAHs RBA values may be explained by the differences in dust or soil properties, with the organic matter an important parameter affecting bioavailability (Li et al., 2016). To test this hypothesis, the organic matter (OM) of indoor dust was measured for their correlation with PAHs RBA. Negative but not significant correlations ($p > 0.05$) were observed between the OM and Phe, Pyr and B(a)p RBA (Fig. S4). Only a significant negative correlation was found between OM and Flu RBA ($r2 = 0.53$, $p < 0.05$). The non-significant correlation observed may be attributed to the relative small sample size, and heterogeneity of OM in indoor dust, leading to the variation of the affinity for PAHs and subsequently resulted in the difference in release of PAHs from dust (Li et al., 2016). Nevertheless, further research should be conducted about the effects of properties of indoor dust on the bioavailability of PAHs.

3.5. Correlation between PAHs RBA and PAHs bioaccessibility

Linear regression analysis was performed to study the relationship between bioaccessibility data with or without sorptive sinks and PAHs RBA values, in order to determine the suitability of *in vitro* assays for a surrogate measure of PAHs RBA (Fig. 3). By establishing significant *in vivo-in vitro* correlations (IVIVC), poor correlation was found between the bioaccessibility using PBET without sorptive sinks for Phe (r^2 = 0.38, p = 0.0604), Flu (r^2 = 0.28, p = 0.1025), Pyr(r^2 = 0.33, p = 0.078) and B(a)p (r^2 = 0.38, p = 0.0618). The results were consistent with previous studies with PAHs in soil samples (James, et al., 2011; Juhasz et al., 2014; Li, et al., 2015). When the sorptive sinks were employed to assist the extraction of PAHs from indoor dust, IVIVC was significantly improved (Fig. 3), which indicated that those sorptive sinks possess potentials to predict the PAHs RBA. However, there was a large difference for sorptive sinks to improve IVIVC through comparing the correlation coefficients. For Phe, the coefficients were decreased in the order of silica (r^2 = 0.80) > polyE (r^2 = 0.72) > tenax (r^2 = 0.57) > C18 (r^2 = 0.53). For Flu, the coefficients were decreased in the order of tenax (r^2 = 0.84) > polyE (r^2 = 0.78) > silica (r^2 = 0.68) > C18 (r^2 = 0.36). For Pyr, the coefficients were decreased in the order of polyE (r^2 = 0.85) > silica (r^2 = 0.84) > tenax (r^2 = 0.57) > C18 (r^2 = 0.47). For

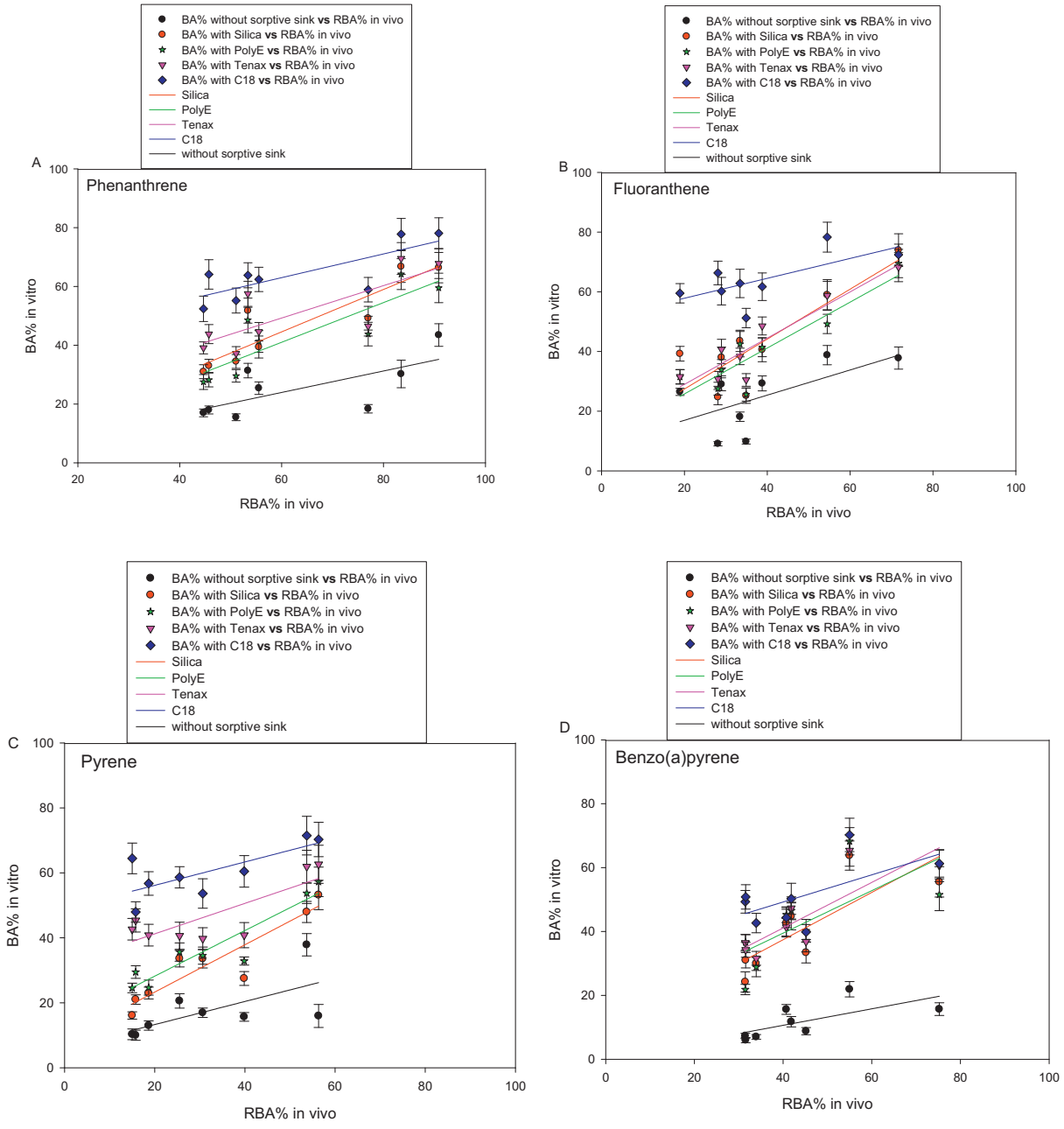

Fig. 3. Comparison of PAHs relative bioavailability and PAHs bioaccessibility of the PBET *in vitro* assays with or without sorptive sinks. Linear regression relationship was used to investigate the correlation between the RBA% *in vivo* and BA% *in vitro*. A: Without sorptive sink: y = 0.365× + 0.020 (r^2 = 0.38, p = 0.0604); Silica: y = 0.721× + 0.012 (r^2 = 0.80, p = 0.0018); PolyE: y = 0.675× + 0.005 (r^2 = 0.72, p = 0.005); Tenax: y = 0.551× + 0.162 (r^2 = 0.57, p = 0.0183); C18: y = 0.404× + 0.39 (r^2 = 0.53, p = 0.024); B: Without sorptive sink: y = 0.422× + 0.085 (r^2 = 0.28, p = 0.1025); Silica: y = 0.836× + 0.107 (r^2 = 0.68, p = 0.0074); PolyE: y = 0.769× + 0.104 (r^2 = 0.78, p = 0.0022); Tenax: y = 0.775× + 0.135 (r^2 = 0.84, p = 0.0009); C18: y = 0.333× + 0.51 (r^2 = 0.36, p = 0.068); C: Without sorptive sink: y = 0.355× + 0.0062 (r^2 = 0.33, p = 0.078); Silica: y = 0.728× + 0.087 (r^2 = 0.83, p = 0.011); PolyE: y = 0.702× + 0.14 (r^2 = 0.85, p = 0.007); Tenax: y = 0.47× + 0.32 (r^2 = 0.57, p = 0.018); C18: y = 0.36× + 0.49 (r^2 = 0.47, p = 0.037); D: Without sorptive sink: y = 0.258× + 0.0029 (r^2 = 0.38, p = 0.0618); Silica: y = 0.741× + 0.078 (r^2 = 0.58, p = 0.0172); PolyE: y = 0.665× + 0.129(r^2 = 0.39, p = 0.059); Tenax: y = 0.713× + 0.126 (r^2 = 0.65, p = 0.0096); C18: y = 0.426× + 0.32 (r^2 = 0.28, p = 0.101).

B(a)p, the coefficients were decreased in the order of tenax ($r^2 = 0.65$) > silica ($r^2 = 0.58$) > polyE ($r^2 = 0.39$) > C18 ($r^2 = 0.28$) (Fig. 3).

There is no guideline for correlation coefficient indicating the acceptable predication of RBA. If $r^2 = 0.6$ was set as the acceptable value for establishing a surrogate approach to measure PAHs RBA, polyE and silica would be the optimum choice, followed by tenax, which was consistent with the results of comparison of sorption kinetics constant. In addition, the slope of polyE and silica are close to 1 (Fig. 3), which indicated that the difference of absolute values between their corresponding bioaccessibility and RBA were minor (Denys et al., 2012). The stronger predictive ability of polyE and silica may be attributed to the fact that they are more likely to mimic the uptake of PAHs by caco-2 (Fig. 2). Though C18 exhibited the strongest ability to enhance the release of PAHs into intestinal juice, it may present a more aggressive PAHs sorption ability than caco-2 cell, and which may subsequently result in overestimation of RBA (James et al., 2011).

The present results indicate that silica and polyE have the potential to simulate caco-2 cell and the inclusion of these materials in the PBET is likely to predict PAHs RBA in indoor dust. However, whether those sorptive sinks are suitable for predicating RBA of other organic pollutants should be further investigated, and the potential use in other animal models and other biological endpoints will require further research.

4. Conclusion

The sorption efficiency of PAHs by sorptive sinks including silica, polyE, tenax, and C18 were compared with that by caco-2 cells based on sorption kinetics assay. The elimination rate constants of phenanthrene, fluoranthene, pyrene, benzo(a)pyrene by caco-2 cell were more closely to that of silica and polyE compared to other materials. This indicated that these materials might be the preferable sorptive sinks to simulate absorption of PAHs by intestinal cells. The bioaccessibility of PAHs in indoor dust ranged from 6.00–43.5%, based on PBET and the sorptive sinks added in the intestinal solution led to 1.17 to 8.47-fold enhancement of bioaccessibility. The results of correlation of *in vivo* PAHs relative bioavailability (RBA) and *in vitro* digestion bioaccessibility indicated that silica and polyE were more likely to predict PAHs RBA of indoor dust, which was consistent with the results of sorption kinetics assay.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.07.102.

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