Functionalized Nanoporous Silica for the Removal of Heavy Metals from Biological Systems; Adsorption and Application

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Supporting Information

ADDITIONAL EXPERIMENTAL INFORMATION

K_d measurements. For K_d measurements, the test solutions were spiked with divalent heavy metal ions (Cd^{2+}, Hg^{2+}, Pb^{2+}) and trivalent arsenic (As^{3+}) to obtain 50 μg/L each. After 30 min of incubation, it was aliquoted into 4.9 mL volumes in a 20 mL polypropylene vial. The solution was then spiked with 0.1 mL of a suspension of solid sorbent and deionized distilled (DI) water at solid per liquid ratio (S/L in the unit of g/L throughout) of 10. This resulted in a final S/L of 0.2. The control was performed in the same fashion but without solid sorbent. The sample was then shaken for 2 hrs at 160 rpm on an orbital shaker. After 2 hrs, the solution was removed by filtering thru 0.45-μm syringe Nylon-membrane filters and the filtrate was kept in 2 vol. % HNO_3 prior to the metal analysis. The metal concentrations in the control (no sorbent) and the test solutions (after being contacted with a sorbent material) were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS, Agilent 7500ce, Agilent Technologies, CA). All batch experiments were performed in triplicate and the average values were reported.

Sorption capacity. The sorption capacity of sorbents for metal ions was measured in the same fashion as with the K_d, but only a single element was used and its concentration was varied in the solution until maximum sorption capacity was obtained. This was accomplished by using a large excess of metal ions to the number of binding sites on the sorbent materials (e.g., 0.1 to 4 mg/L of metal ion at S/L of 0.002).

Sorption kinetics. The kinetics of metal sorption was performed in the same fashion as with the equilibrium studies except that 1 mL of well-mixed aliquot was removed and filtered at 1, 2, 5, 10, 30, 60 min, 2, 8, and 24 hr, and the initial sample volume was increase to 50 mL to minimize the change in S/L due to the sampling.

Metal-prebinding on SH-SAMMS for Caco-2 cell uptake assessment. The metal pre-binding step was performed using 0.1 g of SH-SAMMS in 100 mL of 0.05 M CH_3COONa (pH 6.5) containing 1 mg/L (each) of As^{3+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}. The uptake of each metal on SH-SAMMS was assessed by the difference of metal concentrations in the solutions before and after contacting with SH-SAMMS. Results show that there were 1.0 mg Cd, 1.0 mg Hg, 1.0 mg Pb, and 0.6 mg As per gram of SH-SAMMS. The solution was removed from the suspension and the metal-bound SH-SAMMS was then rinsed with copious amount of deionized water and methanol and air-dried. The solid is then ready to be used with the Caco-2 cells in Transwell® polycarbonate culture insert as shown in Figure 1S.

Fluorescent dye-tagged SH-SAMMS. A 100 mg quantity of SH-SAMMS was dissolved in 5 mL of DMSO. To this suspension, 5 mg of Alexa Fluor 488 dye (Alexa Fluor® 488 C5 maleimide, Invitrogen, CA) was added and the suspension was stirred vigorously for 2 hr at room temperature. The dye-tagged SAMMS were collected *via* vacuum filtration and washed

with copious amount of ethanol. It was then vacuum-dried and stored in the dark at 4 °C prior to use. Analysis on a Horiba Jobin-Yvon Fluorolog Tau 3 spectrofluorometer confirmed attachment of the fluorescent dye by the presence of a strong emission peak at 519 nm followed by excitation at 493 nm.

Caco-2 cell culture. Caco-2 (HTB-37) cell line (ATCC, Manassas, VA) was used in these studies. The cells were grown in a standard cell growth medium (Dulbecco's modified essential medium, DMEM), supplemented with 2 mM L-glutamine, 20% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin, all from Invitrogen (Carlsbad, CA). Cells were grown in 35 mm glass cover-slip bottom plates (WPI, Sarasota, FL), coated with type 1 rat collagen (BD Biosciences, Bedford, MA) following the manufacturer instructions. The cells were grown at 37 °C and 5% CO_2 for one week until reaching ~80% confluence.

Fluorescence and DIC microscopy. Fluorescence laser microscope (Axiovert 200, Zeiss), equipped with a 100X oil-immersion objective (Plan-Apochromat, N.A.=1.4, Zeiss) and a 2 X relay lens in the emission path to the CCD camera were used, leading to a 200X total magnification. A blue laser (Innova, Coherent) was used to excite the fluorescent dye-tagged SAMMS particles at 488 nm. Fluorescence images were acquired by a back-illuminated nitrogen-cooled CCD camera (Spec-10, Roper Scientific, Inc.). DIC images were also acquired at 200X magnification using the same CCD camera.

Table S1. The affinity (K_d) of SH-SAMMS for As, Cd, Hg, and Pb in "protein-free" matrices.

Matrix[a]	Conductivity (mS/cm)	pH	K_d[b] (mL/g) in various matrices			
			As	Cd	Hg	Pb
River Water	0.15	7.66	$7.2x10^4$	$1.0x10^7$	$3.6x10^5$	$5.3x10^6$
Ground Water	0.33	8.00	$5.7x10^4$	$1.1x10^7$	$5.9x10^5$	$5.6x10^6$
Sea Water	45.2	7.74	$9.2x10^4$	$1.5x10^7$	$2.5x10^6$	$3.4x10^6$
0.3 M HNO_3	62.2	0.66	-	0	$3.1x10^5$	$7.2x10^1$

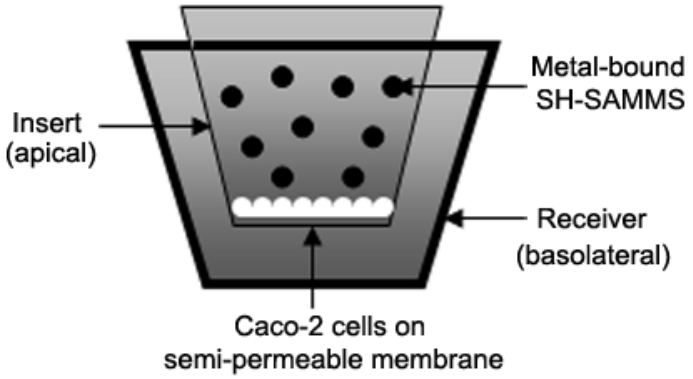

Figure S1. Schematic of Caco-2 cells in a Transwell® polycarbonate culture insert.

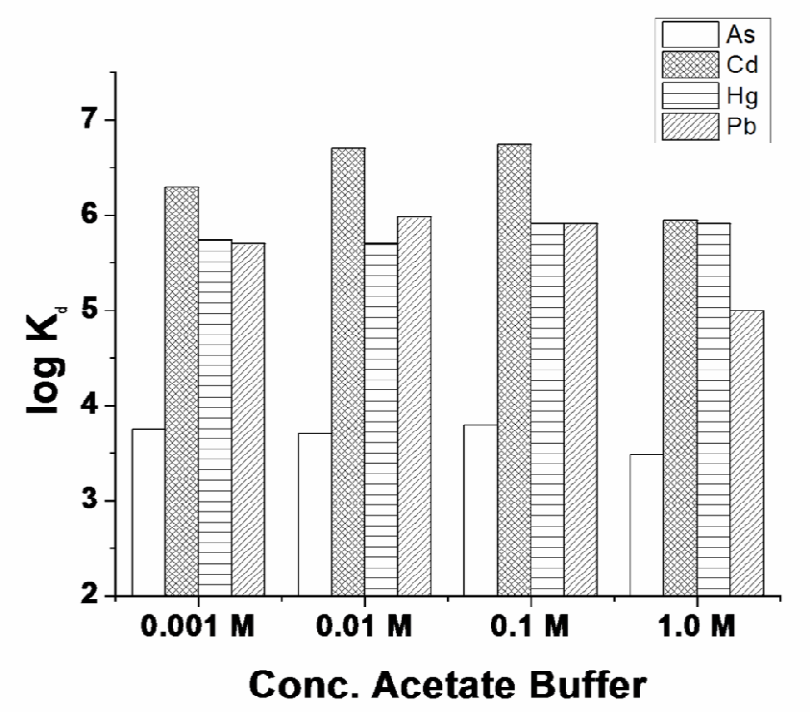

Figure S2. Effect of ionic strength of CH_3COONa (pH 7.3) on the affinity (K_d) of As, Cd, Hg, and Pb adsorption on SH-SAMMS, initial metal ions of 100 µg/L and S/L of 0.2 g/L.

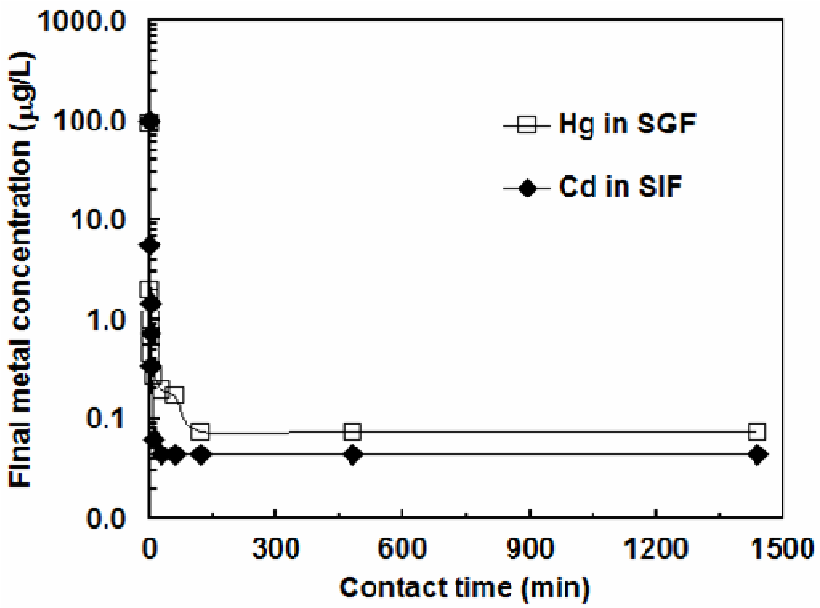

Figure S3. Kinetics of Hg in synthetic gastric fluids (pH 1.12) and Cd in synthetic intestinal fluid (pH 6.80) on SH-SAMMS, initial metal concentration of 100 µg/L, and S/L of 0.2 g/L.

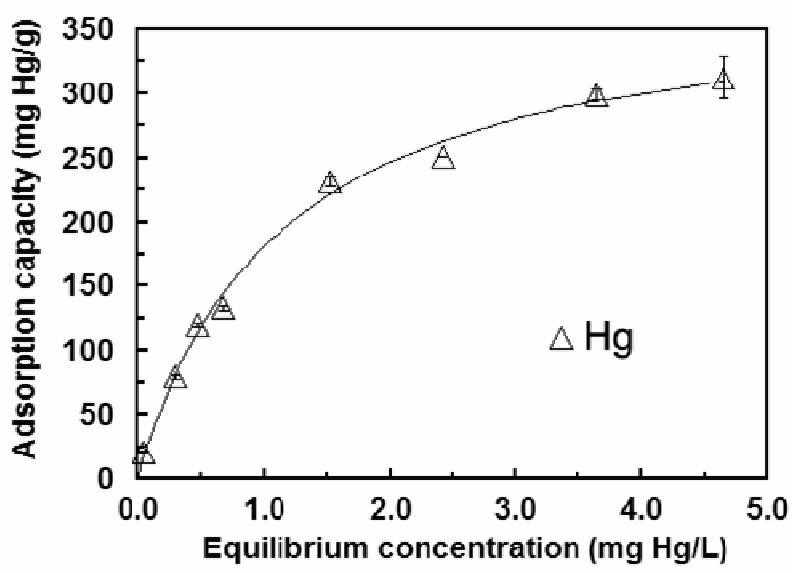

Figure S4. Adsorption isotherm of Hg on SH-SAMMS, measured in synthetic gastric fluid (pH 1.10), S/L of 0.002 g/L, symbols represent data and line represents Langmuir isotherm model.

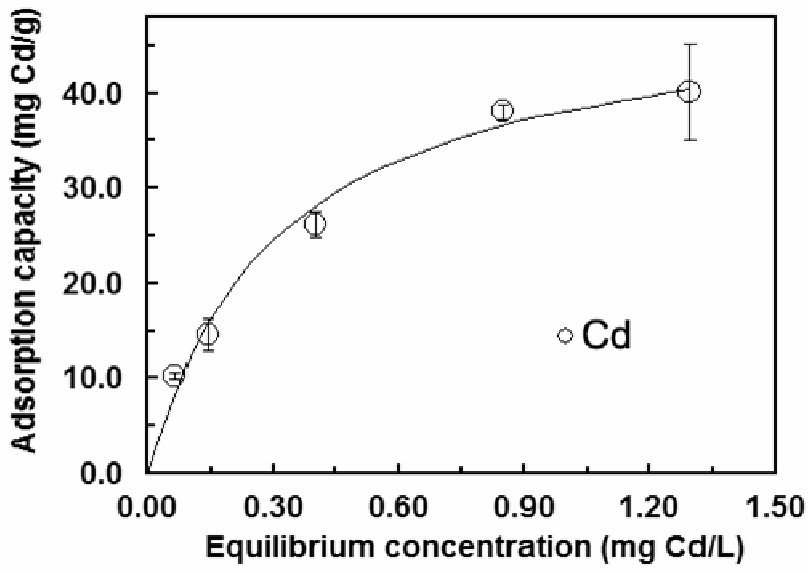

Figure S5. Adsorption isotherm of Cd on SH-SAMMS, measured in synthetic intestinal fluid (pH 6.80), S/L of 0.002 g/L, symbols represent data and line represents Langmuir isotherm model.

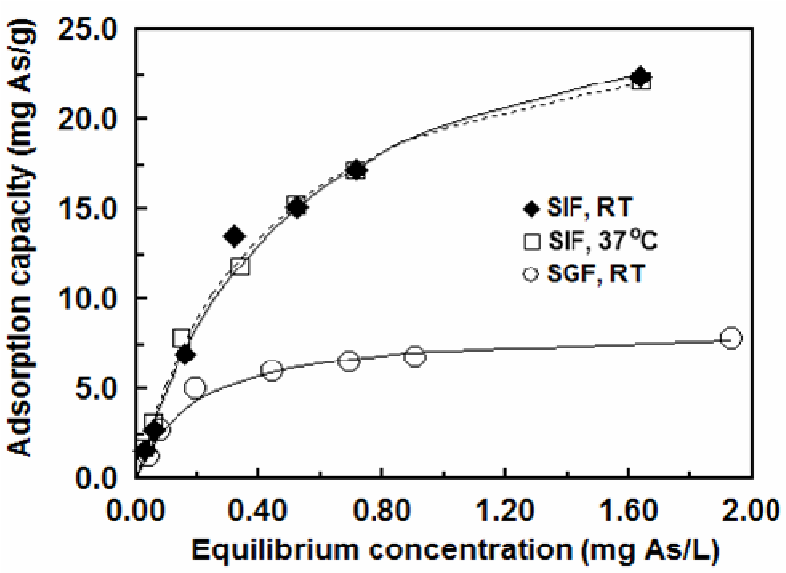

Figure S6. Adsorption isotherms of As(III) on SH-SAMMS, measured in synthetic intestinal fluid (SIF, pH 6.80), and synthetic gastric fluid (SGF, pH 1.10), S/L of 0.01 g/L, symbols represent data and lines represent Langmuir isotherm model.