Paper

# IN VITRO AND IN VIVO EVALUATION OF A NOVEL FERROCYANIDE FUNCTIONALIZED NANOPOUROUS SILICA DECORPORATION AGENT FOR CESIUM IN RATS

Charles Timchalk,* Jeffrey A. Creim,* Vichaya Sukwarotwat,* Robert Wiacek,* R. Shane Addleman,* Glen E. Fryxell,* and Wassana Yantasee†

*Abstract*—**Novel decorporation agents are being developed to protect against radiological terrorist attacks. These sorbents, known as the self-assembled monolayer on mesoporous supports (SAMMS™), are hybrid materials where differing organic moieties are grafted onto mesoporous silica ($SiO_2$). In vitro experiments focused on the evaluation and optimization of SAMMS for capturing radiocesium ($^{137}$Cs); therefore, based on these studies, a ferrocyanide copper (FC-Cu-EDA)-SAMMS was advanced for in vivo evaluation. In vivo experiments were conducted comparing the performance of the SAMMS vs. insoluble Prussian blue. Groups of jugular cannulated rats (4/treatment) were evaluated. Animals in Group I were administered $^{137}$Cs chloride (~40 $\mu$g kg$^{-1}$) by intravenous (i.v.) injection or oral gavage; Group II animals were administered pre-bound $^{137}$Cs-SAMMS or sequential $^{137}$Cs chloride + SAMMS (~61 ng kg$^{-1}$) by oral gavage; and Group III was orally administered $^{137}$Cs chloride (~61 ng kg$^{-1}$) followed by either 0.1 g of SAMMS or Prussian blue. Following dosing, the rats were maintained in metabolism cages for 72 h and blood, urine, and fecal samples were collected for $^{137}$Cs analysis (gamma counting). Rats were then humanely euthanized, and selected tissues analyzed. Orally administered $^{137}$Cs chloride was rapidly and well absorbed (~100% relative to i.v. dose), and the pharmacokinetics (blood, urine, feces, and tissues) were very comparable to the i.v. dose group. For both exposures the urine and feces accounted for 20 and 3% of the dose, respectively. The prebound $^{137}$Cs-SAMMS was retained primarily within the feces (72% of the dose), with ~1.4% detected in the urine, suggesting that the $^{137}$Cs remained tightly bound to SAMMS. SAMMS and Prussian blue both effectively captured available $^{137}$Cs in the gut with feces accounting for 80–88% of the administered dose, while less than 2% was detected in the urine. This study suggests that the functionalized SAMMS outperforms Prussian blue in vitro at low pH, but demonstrates comparable in vivo sequestration efficacy at low exposure concentrations. The comparable response may be the result of the low $^{137}$Cs chloride dose and high sorbent dosage that was utilized. Future studies are planned to optimize the performance of SAMMS in vivo over a broader range of doses and conditions.**

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**Key words: absorption; cesium; chelation; pharmacokinetics**

## INTRODUCTION

Due to recent terrorist events and the resulting detailed assessments of potential threat scenarios, there has been a concerted effort to develop needed medical countermeasures for chemical, biological, and radiological threats. It has been suggested that one of the most plausible scenarios involves a radiological attack. This scenario might entail weapons that produce a fission reaction (improvised nuclear device) or alternatively the scattering of radiological materials (radiological dispersion device, RDD), resulting in exposure and internalization of radioisotopes (Valentin 2005; Cassatt et al. 2008; Tofani and Bartolozzi 2008).

Radiocesium (including $^{137}$Cs) is of particular concern since it is extensively used in industry and medicine and is a nuclear fission product that has a relatively long environmental half-life (Faustino et al. 2008). The pharmacokinetics of $^{137}$Cs has been extensively studied: $^{137}$Cs is well absorbed following inhalation or oral exposure; $^{137}$Cs uniformly distributes within the body and competes with potassium (K) for active and passive membrane transport (Nelson et al. 1961; Chertok and Lake 1973; Gregus and Klaassen 1986; Leggett 1986; Leggett et al. 2003; Le Gall et al. 2006; Cassatt et al. 2008). The major route of $^{137}$Cs excretion is via the urine (~10% of total burden 2 d post-exposure); whereas, excretion via the feces is limited due to intestinal reabsorption following biliary excretion (Nigrovic 1965; Le Gall et al. 2006). Hence, in the absence of decorporation therapy the average biological half-life of $^{137}$Cs in adults and children ranges from 50–150 d and 25–30 d, respectively (Lipsztein et al. 1991; Leggett et al. 2003; Faustino et al. 2008).

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Decorporation agents have the capacity to increase the rate of elimination or excretion of radiocontaminants that have been ingested and/or inhaled and subsequently absorbed into the body. For oral decorporation of cesium and thallium (Tl), Prussian blue is the only drug that is currently U.S. Food and Drug Administration (FDA) approved (Cassatt et al. 2008; Faustino et al. 2008; Yang et al. 2008). In this regard, Prussian blue has been effectively used to treat Cs exposure following the Chernobyl nuclear reactor accident and the Goiânia accident in Brazil (Giese 1988; Assimakopoulos et al. 1991; Ioannides et al. 1991; Melo et al. 1994). After oral administration, insoluble Prussian blue is not absorbed and is readily cleared from the gastrointestinal (GI) tract as a function of transit time. It has been suggested that the binding of Cs and Tl with Prussian blue involves chemical ion exchange, physical adsorption, and ion trapping (Melo et al. 1996; Faustino et al. 2008).

At the Pacific Northwest National Laboratory (PNNL), a new class of nanostructured sorbents, self-assembled monolayer on mesoporous supports (SAMMS™) materials, has been developed to facilitate the cleanup of radionuclides from complex waste found at the U.S. Department of Energy (DOE) sites. SAMMS are hybrid materials constructed from grafting selective organic moieties onto mesoporous silica ($SiO_2$). SAMMS materials are highly efficient and have superior properties over conventional sorbents. Their multi-ligand sequestration ability enhances the binding affinity and stability. Exceptional capacity results from the high surface area of silica substrate (~1,000 $m^2\ g^{-1}$) and the monolayer self-assembly technique that affords a high functional group density up to 10-fold higher than simple functionalization methods (Feng et al. 1997). SAMMS have rigid, open pore structures with suitable pore size that enhances the mass transfer of ions to the binding sites resulting in very rapid capture. The interfacial chemistry of SAMMS has been fine-tuned to selectively sequester specific target species, including lanthanide (Fryxell et al. 2004; Yantasee et al. 2005b), actinide (Fryxell et al. 2000; Birnbaum et al. 2002; Lin et al. 2005; Fryxell et al. 2005), heavy and transition metal ions (Feng et al. 1997; Chen et al. 1999; Yantasee et al. 2003), cesium (Lin et al. 2001), radioiodide (Mattigod et al. 2003), and oxometallate anions (Fryxell et al. 1999).

Our current research has focused on extending the application of SAMMS from their proven utility in environmental clean-up to their potential utility for radionuclide decorporation. In the current manuscript we report on both the in vitro and in vivo (rodent model) efficacy of SAMMS to decorporate $^{137}Cs$ and to initially compare the decorporation capacity relative to Prussian blue.

## MATERIALS AND METHODS

### Cesium (Cs) and radiocesium ($^{137}Cs$)

For in vitro batch experiments cesium under ionic ($Cs^+$) form was purchased as a standard solution at a concentration of 1,000 mg $L^{-1}$ in ~2% $HNO_3$. For in vivo pharmacokinetic evaluation, two separate batches of cesium chloride ($^{137}Cs$) were obtained from Amersham International (Amersham, UK) and ICN Isotope and Nuclear Division (Irvine, CA). The Amersham International $^{137}Cs$ chloride stock radiological activity was 46.6 kBq $mL^{-1}$ in 0.1 M HCl, while the ICN stock chemical composition was 1,132 MBq $mL^{-1}$ in 0.5 M HCl.

**Sorbents.** The synthesis of FC-Cu-EDA-SAMMS sorbent has been described previously (Lin et al. 2001). Prussian blue, $Fe_4[Fe(CN)_6]_3$, was purchased from Aldrich Co (St. Louis, MO). Fig. 1 illustrates the chemical structures of the FC-Cu-EDA-SAMMS and Prussian blue.

### Gamma counting

Samples were counted for 10 min each using a shielded, well type gamma counter (Wallac 1480 WIZARD®; PerkinElmer, Waltham, MA). The counting efficiency for $^{137}Cs$ was 47% with minimal sample crosstalk (0.001%).

### In vitro experimental design

***$K_d$* measurements.** The metal sorption performance of SAMMS and Prussian blue was evaluated in terms of the distribution coefficient ($K_d$, mL $g^{-1}$), which is a mass-weighted partition coefficient between solid phase and liquid supernatant phase. Two test matrices were synthetic gastric fluid, which contained 0.03 M NaCl, 0.085 M HCl, and 0.32% (w/v) pepsin, and were prepared daily following the recommendations of the U.S.

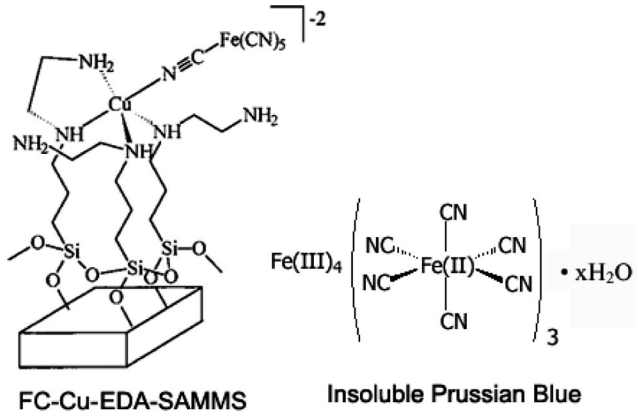


**Fig. 1.** Chemical structures of FC-Cu-EDA-SAMMS and Prussian blue.

Pharmacopeia for drug dissolution studies in stomach (USP 1990), while the synthetic intestinal fluid, which contained 0.05 M $NaHCO_3$, has been used as intestinal fluid simulant by other work (Hamel et al. 1999; Ellickson et al. 2001). The $K_d$ values of Cs in synthetic gastric and intestinal fluid were measured in batch experiments with 50 ng $mL^{-1}$ starting concentration of Cs and liquid per solid (L/S) ratio of 5,000 mL per gram of material. The suspension was shaken in a polypropylene bottle at a speed of 250 rpm for 2 h at 37°C. After the batch contacts, the metal-laden sorbents were filtered through a 0.2 $\mu$m Nylon filter in a polypropylene housing. Both initial and final solutions (before and after the batch experiments) were analyzed by an inductively coupled plasma-mass spectrometer (ICP-MS, Agilent 7500ce; Agilent Technologies, Santa Clara, CA). The measurements were carried out in triplicate and the average values were reported.

**Sorption isotherms.** The sorption capacities of SAMMS and Prussian blue for metal ions were measured in the same fashion as with the $K_d$, but the starting concentrations of Cs were 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 5 mg $L^{-1}$ of Cs at L/S of 10,000 mL $g^{-1}$).

### In vivo experimental design

**Animals.** All animal procedures described in the present study were conducted in accordance with the guidelines for the care and use of laboratory animals in the National Institutes of Health/National Research Council (NIH/NRC) Guide and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee (IACUC) of Battelle, Pacific Northwest Division. For all studies, male Sprague-Dawley rats (291–341 g) with jugular vein cannulae were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Rats were housed in plastic metabolism cages and were fed Purina Certified Rodent Chow® 5002 (Purina Mills, St. Louis, MO) ad libitum. Water was likewise available ad libitum throughout the duration of the study. Blood was collected through the jugular vein cannula at 0.5, 1, 2, 3, 6, 12, 24, 48, and 72 h post-dosing. Urine and feces were collected continuously, and sample collections were accumulated for 24, 48, and 72 h post-dosing. All rats were euthanized at 72 h post-dosing and selected tissues were collected for analysis.

**Treatment groups and dosing.** Three experimental groups were evaluated. Group I (controls) received only $^{137}$Cs chloride by intravenous (i.v.) or oral administrations and was used to establish the oral bioavailability and clearance rate for $^{137}$Cs. Group II established the stability of the $^{137}$Cs-SAMMS adduct (pre-bound) and the rate of $^{137}$Cs sequestration in vivo in the rat gut. Group III compared the initial efficacy of SAMMS vs. Prussian blue to sequester $^{137}$Cs following oral exposures.

The $^{137}$Cs chloride stock solutions were initially diluted to an acidic concentration of 0.01 M HCl, then buffered with phosphate buffered saline (PBS) to make the dosing solutions. ICP-MS analysis of the dosing solutions indicated Cs concentrations of 58.3 $\mu$g $mL^{-1}$, 51.0 ng $mL^{-1}$ and 53.2 ng $mL^{-1}$ for Groups I, II, and III, respectively. The radiological activity of these dosing solutions by gamma count was 8.14, 18.5, and 17.8 kBq $mL^{-1}$, respectively. The average amount of $^{137}$Cs and associated radioactivity administered to the rats for treatment Group I was 40.4 $\mu$g $kg^{-1}$ and 5.5 kBq $kg^{-1}$, respectively. Whereas, for treatment Groups II and III the average $^{137}$Cs dose was ~61 ng $kg^{-1}$, while the average amount of radioactivity administered was 22.6 and 20.4 kBq $kg^{-1}$, respectively. For Group II, the pre-bound $^{137}$Cs-SAMMS was prepared by mixing the $^{137}$Cs dose solution with an excess of SAMMS and allowing the solution to mix for 30 min at room temperature. The SAMMS was then filtered and the remaining supernatant was analyzed for radioactivity—which was at background levels (data not shown), indicating that all the $^{137}$Cs was bound to the SAMMS. The pre-bound $^{137}$Cs-SAMMS was then orally administered to rats as previously described. For Group III, 0.1 g of SAMMS or Prussian blue was suspended in 1 mL of PBS which was administered within 1–2 min of the $^{137}$Cs chloride to rats by gavage.

**Data analysis.** The time-course of $^{137}$Cs was analyzed using non-compartmental methods. Peak concentrations of $^{137}$Cs in blood ($C_{max}$) were determined by a visual analysis of the individual observed concentration-time data. The area under the blood concentration-time curve from 0–72 h (area-under-the-curve, AUC) was determined using GraphPad Prism®4 (GraphPad Software, La Jolla, CA) using the trapezoidal rule. Other than the calculation of mean ± standard deviation (SD), no additional statistical evaluations were conducted.

## RESULTS

### In vitro

In this study, the sorption performance of ferrocyanide copper (II) immobilized on mesoporous silica (FC-Cu-EDA-SAMMS) for Cs in a gastric and intestinal fluid simulant was evaluated in terms of adsorption affinity

and capacity. The performance was also evaluated against Prussian blue.

**Adsorption affinity.** The adsorption affinity of Cs on SAMMS and Prussian blue has been investigated using synthetic gastric and intestinal fluid matrix simulants (Table 1). The sorption affinity is often represented in term of the distribution coefficient, $K_d$ (in units of mL $g^{-1}$), which is calculated as:

$$K_d = \frac{(C_o - C_f)}{C_f} \times \frac{V}{M}, \quad (1)$$

where $C_o$ and $C_f$ are the initial and final concentrations in the solution of the target species determined by ICP-MS, $V$ is the volume of solution (mL), and $M$ is the mass of material (g). The distribution coefficient expresses the chemical binding affinity of target metal ion to a sorbent under the conditions tested. The in vitro measured $K_d$ for the SAMMS substantially exceeded the adsorption affinity of Prussian blue in simulants of gastric (~29-fold) and intestinal fluid (~3-fold). These results indicate that the SAMMS material has excellent affinity for the Cs and exceeded the affinity of Prussian blue under these in vitro experimental conditions.

**Adsorption capacity.** The adsorption isotherms on both sorbents are shown in Figs. 2 and 3 for Cs in gastric and intestinal fluid simulants, respectively, and the calculated maximum capacity is presented in Table 1. These adsorption isotherms were measured by increasing the loading of Cs in the simulants onto SAMMS or Prussian blue while maintaining L/S ratio of 10,000 mL $g^{-1}$. The plot between the equilibrium sorption capacities vs. solution metal concentrations represents the adsorption isotherm curve. The sorption isotherm data can be fitted to the Langmuir adsorption model, which is given by:

$$Q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e}, \quad (2)$$

where $Q_{max}$ is the adsorption capacity (mg of metal ion $g^{-1}$ of sorbent) when all adsorption sites are occupied,

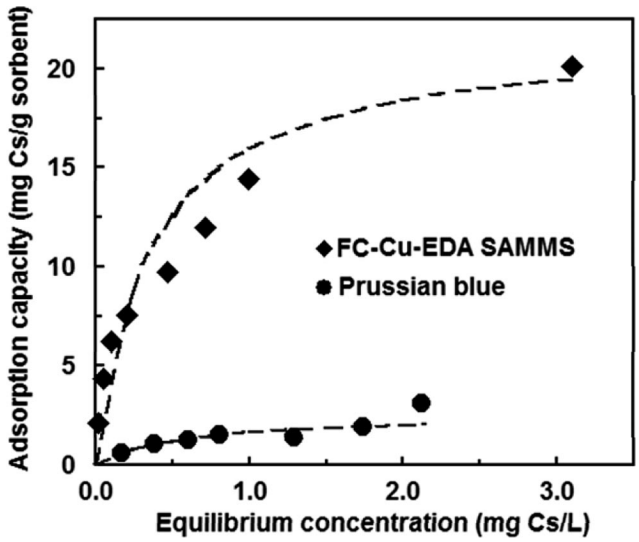


**Fig. 2.** Cs adsorption capacity of FC-Cu-EDA-SAMMS and Prussian blue, measured in synthetic gastric fluid (pH 1.1), L/S of 10,000 mL $g^{-1}$. Dashed lines represent Langmuir isotherm models.

$C_e$ is the equilibrium concentration of the metal ion, and the Langmuir constant $K_L$ (L of solution $mg^{-1}$ of metal ion) represents the ratio of the adsorption rate constant to the desorption rate constant. For both matrices adsorption isotherm data on both materials were in agreement with the Langmuir model with an excellent fit ($R^2 > 0.99$), indicating monolayer adsorption (without precipitation) of Cs ions. The maximum sorption capacities for Cs with SAMMS or Prussian blue using gastric or intestinal fluid matrix simulants as estimated from the Langmuir model are listed in Table 1. In gastric fluid simulant at low pH (1.1), the SAMMS exhibited a very high maximum sorption capacity exceeding Prussian blue by an order of magnitude (21.7 vs. 2.6 mg Cs $g^{-1}$, respectively ~10-fold); whereas in the intestinal fluid simulant (pH 8.6) SAMMS and Prussian blue had a similar capacity (17.9 and 16.5 mg Cs $g^{-1}$, respectively ~1.1-fold).

**Table 1.** Adsorption affinity ($K_d$) and maximum sorption capacity of Cs, measured on FC-Cu-EDA-SAMMS (SAMMS) and Prussian blue in synthetic gastric and intestinal fluids.

| | $K_d$ (mL $g^{-1}$)[a] | | Max. capacity (mg Cs $g^{-1}$)[b] | |
|---|---|---|---|---|
| Matrix | SAMMS | Prussian blue | SAMMS | Prussian blue |
| Synthetic gastric fluid, pH 1.1 | 156,000 ± 46,000 | 5,400 ± 490 | 21.7 | 2.60 |
| Synthetic intestinal fluid, pH 8.6 | 230,000 ± 24,000 | 73,000 ± 22,000 | 17.9 | 16.5 |

[a] Measured with initial Cs concentration of 50 ppb and liquid per solid (L/S) ratio of 5,000 mL $g^{-1}$. Values are mean ± SD for 3 determinations.
[b] Estimated from Langmuir adsorption isotherm model of the adsorption data (see Figs. 2 and 3), measured at L/S of 10,000 mL $g^{-1}$ and Cs concentration varied from 0 to 5 ppm; values reported as average of three replicates.

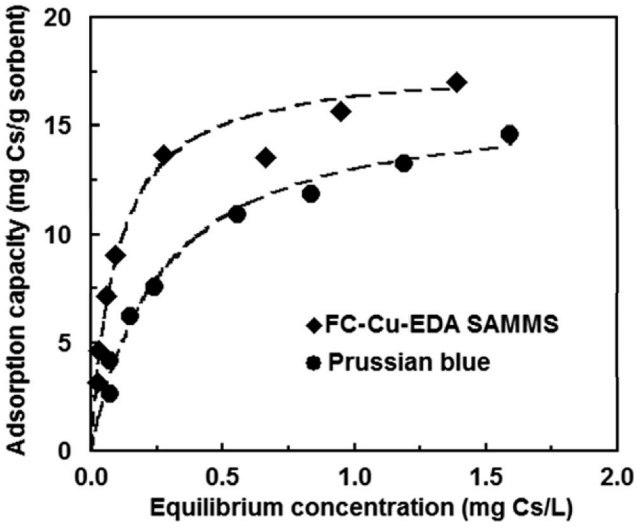


**Fig. 3.** Cs adsorption capacity of Cu-FC-EDA-SAMMS and Prussian blue, measured in synthetic intestinal fluid (pH 8.6), L/S of 10,000 mL $g^{-1}$. Dashed lines represent Langmuir isotherm models.

**In vivo**

The pharmacokinetics of $^{137}$Cs uptake, distribution, and elimination were evaluated in rats following single dose exposures to $^{137}$Cs chloride (oral and i.v.), both in the presence or absence of decorporation agents (SAMMS and Prussian blue). For all treatment groups (I→III), the time-course of $^{137}$Cs in blood, selected tissues, excreta and calculated AUC are presented in Figs. 4–7 and Table 2. Two differing radiological stocks of $^{137}$Cs chloride were utilized in the current study resulting in substantially different doses (~600-fold difference) of $^{137}$Cs chloride being administered to Group I vs. Groups II and III (40 $\mu$g $kg^{-1}$ vs. 60 ng $kg^{-1}$, respectively). To facilitate comparisons, several additional rats (Groups II and III) were orally administered $^{137}$Cs chloride (w/no decorporation agent) at the low dose (60 ng $kg^{-1}$), and the pharmacokinetics, tissue distribution and urinary and fecal excretion were evaluated. The results were directly comparable to those obtained following the oral (Group I) $^{137}$Cs chloride 40 $\mu$g $kg^{-1}$ dose (data not shown); hence, it was feasible to directly compare the Group I results with Groups II and III to

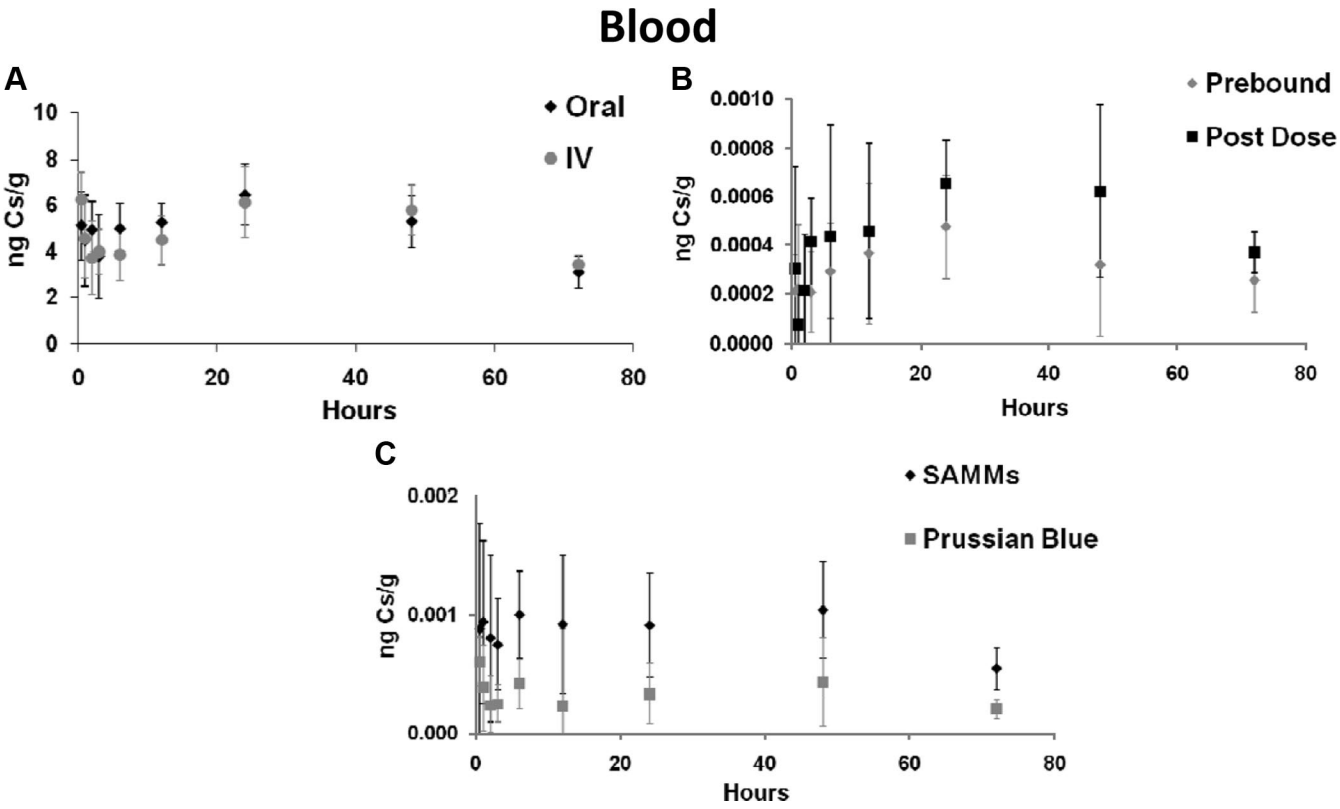


**Fig. 4.** Time-course of $^{137}$Cs in blood of rats measured through 72 h post-dosing. (A) Group I: time-course following oral vs. intravenous (i.v.) administration of $^{137}$Cs chloride; (B) Group II: time-course of $^{137}$Cs following oral gavage administration of prebound $^{137}$Cs-SAMMS vs. co-administration of $^{137}$Cs chloride + SAMMS (post-dose); (C) Group III: time-course of orally administered (gavage) $^{137}$Cs, in which equal amounts (0.1 g) of SAMMS vs. Prussian blue were likewise orally administered by gavage. The values represent the mean ± SD for 4 animals per treatment group. Within each treatment group the rats were administered equal molar doses of $^{137}$Cs.

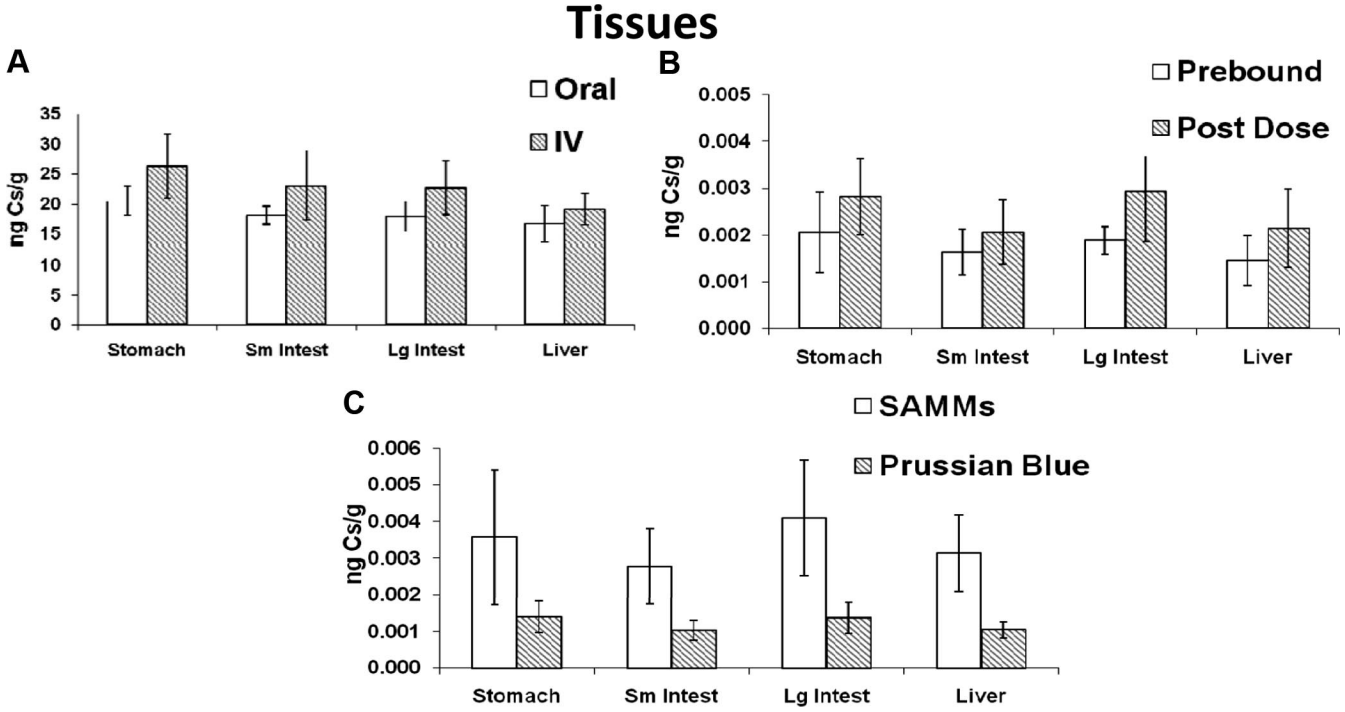


**Fig. 5.** Concentration of $^{137}$Cs in selected tissues obtained from rats at 72 h post-dosing. (A) Group I: equal molar doses of $^{137}$Cs chloride administered by oral gavage or intravenously (i.v.); (B) Group II: tissue concentration of $^{137}$Cs following oral gavage administration of prebound $^{137}$Cs-SAMMS vs. co-administration of $^{137}$Cs chloride + SAMMS (post-dose); (C) Group III: tissue concentration of orally administered $^{137}$Cs chloride in which equal amounts (0.1 g) of SAMMS vs. Prussian blue were likewise orally administered by gavage. The values represent the mean ± SD for 4 animals per treatment group. Within each treatment group the rats were administered equal molar doses of $^{137}$Cs.

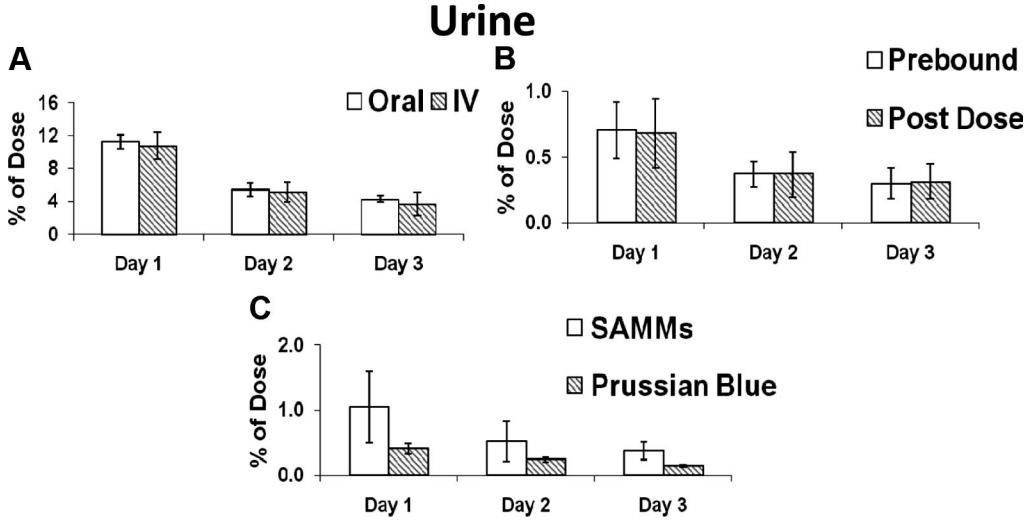


**Fig. 6.** The daily excretion (24 h) of $^{137}$Cs in urine expressed as a percent of administered dose in rats. (A) Group I: equal molar doses of $^{137}$Cs chloride administered by oral gavage or intravenously (i.v.); (B) Group II: urinary $^{137}$Cs excretion following oral gavage administration of prebound $^{137}$Cs-SAMMS vs. co-administration of $^{137}$Cs chloride + SAMMS (post-dose); (C) Group III: urinary $^{137}$Cs excretion of orally administered $^{137}$Cs chloride in which equal amounts (0.1 g) of SAMMS vs. Prussian blue were likewise orally administered by gavage. The values represent the mean ± SD for 4 animals per treatment group. Within each treatment group the rats were administered equal molar doses of $^{137}$Cs.

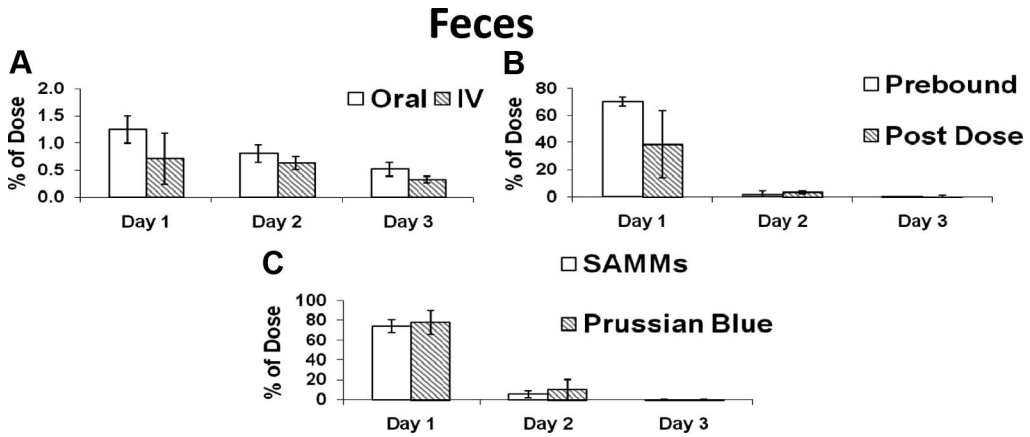


**Fig. 7.** The daily excretion (24 h) of $^{137}$Cs in feces expressed as a percent of administered dose in rats. (A) Group I: equal molar doses of $^{137}$Cs chloride administered by oral gavage or intravenously (i.v.); (B) Group II: fecal $^{137}$Cs excretion following oral gavage administration of prebound $^{137}$Cs-SAMMS vs. co-administration of $^{137}$Cs chloride + SAMMS (post-dose); (C) Group III: fecal $^{137}$Cs excretion of orally administered $^{137}$Cs chloride in which equal amounts (0.1 g) of SAMMS vs. Prussian blue were likewise orally administered by gavage. The values represent the mean ± SD for 4 animals per treatment group. Within each treatment group the rats were administered equal molar doses of $^{137}$Cs.

**Table 2.** Estimated area-under-the concentration (AUC) curve for $^{137}$Cs (radioactivity) quantified in the blood of rats. Group I was administered $^{137}$Cs chloride (~40 $\mu$g kg$^{-1}$) by intravenous (i.v.) injection and oral gavage; Group II administered prebound SAMMS + $^{137}$Cs and SAMMS immediately followed by $^{137}$Cs chloride by oral gavage; and Group III evaluated orally administered $^{137}$Cs chloride (~60 ng kg$^{-1}$) followed by 0.1 g of either SAMMS or Prussian blue.

| | Group I | | | Group II | | Group III | | |
|---|---|---|---|---|---|---|---|---|
| | Oral $^{137}$Cs | i.v. $^{137}$Cs | $^{137}$Cs-SAMMS | SAMMS + $^{137}$Cs | Oral $^{137}$Cs | SAMMS + $^{137}$Cs | Prussian blue + $^{137}$Cs | Oral $^{137}$Cs |
| AUC (ng/g/h) | 366 | 365 | 0.025 | 0.039 | 0.276 | 0.064 | 0.029 | 0.737 |
| Relative AUC ratio[a] | 100% | — | 9% | 14% | — | 9% | 4% | — |

[a] For Group I the AUC ratio (expressed as a %) was based on i.v./oral ratio, for Groups II and III the treatments were compared to the concurrent oral AUC (i.e. Group II $^{137}$Cs-SAMMS/oral $^{137}$Cs).

estimate oral bioavailability for both SAMMS and Prussian blue decorporation.

**Group I.** An evaluation of the pharmacokinetics following the equal molar $^{137}$Cs doses via oral or i.v. administration strongly suggest that the kinetics are very comparable (Fig. 4a). For both dose routes, peak blood concentrations were observed at 0.5 h and 24 h post-dosing, which then gradually declined. The calculated AUC for the oral and i.v. groups are essentially the same (365–366 ng g$^{-1}$ h$^{-1}$), which is consistent with the rapid and complete oral bioavailability of $^{137}$Cs (Table 2). A comparison of the $^{137}$Cs concentration in the GI tract associated tissues/organs at 72 h post-dosing are presented in Fig. 5a. The concentration of $^{137}$Cs was very comparable in the stomach, small and large intestines, and liver, with oral administration resulting in a slightly lower tissue concentration (~78–88%), relative to i.v. administration. The excretion time-course of $^{137}$Cs in urine and feces are very comparable for the oral and i.v. doses and the results are presented in Figs. 6a and 7a. For both exposure routes, the urine is the predominant excretion pathway accounting for 18–20% of the dose; whereas, the feces only accounts for 2–3% (72 h post-dosing). For both excretion pathways the first 24-h collection interval (Day 1) accounted for the majority of $^{137}$Cs that was excreted.

**Group II.** In these experiments equal molar doses of $^{137}$Cs were administered to rats either pre-bound to SAMMS or the SAMMS was sequentially administered following the oral dose of $^{137}$Cs chloride. In addition, to facilitate comparison a single rat was administered $^{137}$Cs only (no SAMMS) (data not shown). The time-course of $^{137}$Cs in the blood and the calculated AUC are presented in Fig. 4b and Table 2. Although the current study did not evaluate any clinically relevant toxicity endpoints, all animals that were administered the SAMMS treatments appeared healthy throughout the course of this study. $^{137}$Cs was detected in the blood following either SAMMS

treatment; however, the peak concentrations (24 h post-dosing) range from 6- to 8-fold lower than what is observed for $^{137}$Cs chloride only. A comparison of the blood $^{137}$Cs AUC suggests that 9 and 14% of the $^{137}$Cs from the pre-bound and sequential SAMMS were absorbed, respectively. A comparison of the $^{137}$Cs concentration in GI tract associated tissues/organs at 72 h post-dosing are presented in Fig. 5b. Consistent with the observed blood time-course results, the tissue concentration of $^{137}$Cs was ~10-fold lower for rats administered the pre-bound and sequential SAMMS, relative to the $^{137}$Cs only. Following the SAMMS administrations (pre-bound and sequential), less than 1.5% of the administered dose of $^{137}$Cs was accounted for in the urine of rats (through 72 h post-dosing); whereas, for the $^{137}$Cs only treatment, the urine accounted for >11% of the administered dose. In contrast, the pre-bound and sequential SAMMS treatments resulted in substantially more fecal excretion of $^{137}$Cs, particularly in the first 24 h where pre-bound and sequential administration accounted for 70 and 39% of the dose, respectively. In comparison, less than 0.5% of the $^{137}$Cs only dose was accounted for in the feces over the same collection interval. These results suggest that SAMMS binds rapidly with available $^{137}$Cs in the gut, and once the $^{137}$Cs is bound it is stable and readily excreted in the feces.

**Group III.** In these experiments rats were orally administered equal molar doses of $^{137}$Cs chloride, then sequentially administered an oral dose (0.1 g) of either SAMMS or Prussian blue and the pharmacokinetics of $^{137}$Cs was evaluated. Again, to facilitate comparisons a single rat was administered $^{137}$Cs only (no SAMMS or Prussian blue) (data not shown). The time-course of $^{137}$Cs in the blood and the calculated AUC are presented in Fig. 4c and Table 2. Both decorporation agents substantially decreased the $^{137}$Cs blood concentration (10- to 100-fold) relative to $^{137}$Cs only. Based on the blood time-course results and the calculated AUC, only 4% of the $^{137}$Cs dose was absorbed following the Prussian blue treatment, while SAMMS resulted in 9% absorption. The tissue concentrations of $^{137}$Cs at 72 h post-dosing are presented in Fig. 5c, and the tissue levels ranged from 20- to 60-fold less than what is observed following the $^{137}$Cs only dose. In the absence of any decorporation agents the total amount of $^{137}$Cs that was cumulatively excreted in the urine over 72 h post-dosing was ~20%; however, when either SAMMS or Prussian blue were administered the total amount of radioactivity that was excreted in the urine was <2% (Fig. 6c). Consistent with the lack of urinary $^{137}$Cs excretion was an increase in the amount of radioactivity eliminated via the feces following SAMMS or Prussian blue decorporation (Fig. 7c). Specifically, an average of 80–90% of the $^{137}$Cs was eliminated via the feces, with the majority (74–78%) within the first 24 h post-dosing for both decorporation agents. These results indicate that SAMMS can effectively decorporate $^{137}$Cs when sequentially administered orally, and the in vivo efficacy of SAMMS (under the conditions of the current evaluation) is reasonably comparable to the current "gold standard" Prussian blue.

## DISCUSSION

The primary objective of the current study was to conduct a preliminary evaluation (in vitro and in vivo) of a new nanostructured sorbents agent (SAMMS) for use in $^{137}$Cs decorporation and provide an initial comparison with Prussian blue. As previously noted, SAMMS are hybrid materials constructed from grafting selective organic moieties onto mesoporous silica ($SiO_2$). For $^{137}$Cs decorporation an FC-Cu-EDA-SAMMS sorbent was synthesized as described previously (Lin et al. 2001). Prussian blue is the active ingredient in the currently FDA approved medical countermeasure for Cs or Tl internal contamination and goes by the trade name Radiogardase® (Faustino et al. 2008).

As evaluated and discussed by Faustino et al. (2008), a number of physiochemical factors (i.e., pH, exposure time, temperature, drying content and particle size) can play important roles in the clinical efficacy of decorporation agents such as Prussian blue and SAMMS. Of significant concern is the potential effect of low pH within the stomach. In this regard, it has been demonstrated that low pH can have a negative effect on the Prussian blue binding of $^{137}$Cs; however, the binding capacity of Prussian blue rapidly recovers with increasing pH and maximum binding capacity is achieved within 4 h at pH 5 (Faustino et al. 2008). The impact of pH is of particular relevance since the transit time in the human stomach is 1–2 h with a pH varying between 1 to 3.5 (oxidizing conditions), and 3–4 h in the small intestine with a pH varying between 5 to 8 (reducing conditions) (ICRP 2006). The findings in the current study (Table 1) that evaluated the absorption affinity ($K_d$) and capacity of Cs with Prussian blue using gastric (pH 1.1) and intestinal (pH 8.6) fluid simulants likewise suggest that binding capacity of Prussian blue is substantially decreased at low vs. high pH (2.6 vs. 16.5 mg Cs $g^{-1}$, respectively). In contrast, the maximum capacity of the SAMMS (22 vs. 18 mg Cs $g^{-1}$) is not substantially impacted by pH. In the case of Prussian blue it has been suggested that Cs binding is reduced at low pH due to the greater availability of hydronium ($H_3O^+$) ions, which compete with $Cs^+$ ions for binding in the Prussian blue lattice (Faustino et al. 2008). In contrast, pH has little

impact on the maximum binding capacity of SAMMS (Table 1), suggesting that the FC-Cu-SAMMS is not protonated to the degree that Prussian blue is at the low pH that is encountered in the stomach.

The design of the current in vivo $^{137}$Cs pharmacokinetic study focused on the capacity of SAMMS to effectively sequester $^{137}$Cs in the upper intestinal tract (stomach and small intestines), since the experimental design entailed a near immediate co-exposure to the radionuclide and SAMMS. Although this experimental design facilitated a rapid in vivo evaluation of efficacy, it doesn't fully assess the ability of SAMMS to capture $^{137}$Cs that has been excreted into the bile. In this case the in vivo decorporation capacity of SAMMS needs to be evaluated following a repeated decorporation strategy similar to the protocol described by Le Gall et al. (2006) to compare the efficacy of Prussian blue and apple-pectin in the rat.

In the current in vivo rodent studies, we have established that orally administered $^{137}$Cs is well absorbed and the pharmacokinetic profile of uptake, distribution and excretion are nearly identical with results obtained with an equal molar i.v. dose. These findings are consistent with previous studies in rats (Nigrovic 1965; Thomas and Thomas 1968; Gregus and Klaassen 1986; Le Gall et al. 2006) and were utilized to justify use of oral $^{137}$Cs administration (without decorporation) for comparison with the sequestration experiments (Groups II and III). The current study has established that SAMMS can rapidly decorporate $^{137}$Cs following oral administration and the SAMMS-$^{137}$Cs complex is very stable in the GI tract. These findings are the first to establish the binding stability of SAMMS in vivo in the GI tract (low–high pH), and are consistent with recently reported results (Yantasee et al. 2009) for a number of functionalized SAMMS that have been developed for lanthanide sequestration under both acidic and alkaline conditions.

Based on the in vitro comparison of SAMMS vs. Prussian blue using the gastric and intestinal simulants, it could be anticipated that SAMMS would outperform Prussian blue in vivo. However, the current in vivo results suggest that the SAMMS performance is approximately (under the current experimental design) equivalent to Prussian blue. As demonstrated by the performance in vitro (Table 1), both materials are very good sorbents and the similar in vivo performance may simply be a result of both sorbents materials being capable of capturing all Cs they encountered during the course of the test. Hence, more stringent testing conditions are needed to more fully characterize the performance similarities and differences. We believe the Fe-Cu-EDA-SAMMS material has not yet been optimized to function up to its potential in vivo. In this regard, we have previously exploited SAMMS and related technologies as biological monitoring sensor systems utilizing a range of biological matrices including saliva, plasma, blood and urine (Yantasee et al. 2005a, 2007). One of the major challenges has been the need to optimize the performance of the materials in the exact matrix that the materials will be evaluated. In the current study, the in vitro gastric and intestinal simulants do not fully mimic the complex physical and chemical complex of the gastric intestinal content which dynamically changes during GI tract transit; hence, it is not unexpected that it is not fully predictive of in vivo performance. Ongoing efforts are currently optimizing SAMMS material, initially focusing on increasing the pore size to maximize access of the functional groups to $^{137}$Cs in the GI tract content matrix. Preliminary results suggest that in a high protein sample there is a 3-fold increase in $K_d$ with a 3-fold increase in pore size of $SiO_2$ (data not shown). Installation of secondary surface chemistry to reduce biofouling and engineering of the particulate morphology may also offer routes to improve sorbent efficacy. Consequently, future in vivo studies are planned to evaluate the performance of optimized SAMMS materials for $^{137}$Cs decorporation relative to Prussian blue using dose-dependent and time delayed studies.

## CONCLUSION

The current study provides the first in vivo evaluation of a new class of nanostructured sorbents (SAMMS) for decorporation of radiocesium in the gastrointestinal tract. Orally administered $^{137}$Cs was rapidly and well absorbed, and the pharmacokinetics were very comparable to what was observed following i.v. administration. Following oral exposure to $^{137}$Cs chloride, sequential dosing with SAMMS rapidly and effectively complexes with available $^{137}$Cs in the gut, thereby enhancing fecal excretion. This study suggests that the functionalized SAMMS outperforms Prussian blue in vitro at low pH, but demonstrates comparable in vivo capture efficacy at low exposure concentrations. Future studies are planned to optimize FC-Cu-EDA-SAMMS structure and chemistry and explore in vivo performance over a broader range of doses and conditions.

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