Electronic Supplementary Information for:

Investigation of Sequential and Enzymatic Extraction of Arsenic from Drinking Water Distribution Solids using ICP-MS

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Experimental

Reagents

The liquid chromatography (LC) mobile phase consisted of A.C.S. Certified glacial acetic acid [CH_3COOH] (Fisher Scientific, Fair Lawn, NJ), ammonium nitrate [NH_4NO_3] (Fisher Scientific), ammonium hydroxide [NH_4OH] (Trace Metal Grade, Fisher Scientific) and ethylenedinitrilotetraacetic acid tetrasodium salt dehydrate [EDTA ($(NaOCOCH_2)_2NCH_2CH_2N(CH_2COONa)_2.2H_2O$] (Baker Analyzed, J.T. Baker Chemical Co., Phillipsburg NJ). A.C.S. Certified magnesium chloride and sodium phosphate monobasic [NaH_2PO_4] (Fisher Scientific) were used to estimate the exchangeable arsenic. The ammonium oxalate [$(NH_4)_2C_2O_4$] (Fisher Scientific) was used to estimate the arsenic bound to the amorphous iron oxide/hydroxide. The Ti-citrate extraction fluid used to estimate the crystalline bound arsenic was prepared from the following reagents: titanium chloride ($TiCl_2$, 99.99%, Aldrich, Milwaukee, WI), sodium citrate ($Na_3C_6H_5O_7$, Certified), EDTA (see above), sodium bicarbonate ($NaHCO_3$, Certified), and sodium hydroxide (NaOH, Certified A.C.S.) All reagents for the Ti-citrate extraction fluid were purchased from Fisher Scientific except for the $TiCl_2$. The Ti-citrate solution was prepared in an inert atomsphere inside an atmospheric bag (Atmosbag, Aldrich). Special safety precautions should be utilized in the preparation of this solution.[1,2] The A.C.S. Certified nitric acid [HNO_3] was purchased from Fisher Scientific (Pittsburgh, PA). The following reagents were used in the synthetic stomach / intestine extraction: pancreatin (from porcine pancreas), bile extract (porcine), pepsin (from porcine stomach mucosa), sodium bicarbonate ($NaHCO_3$ 99.5%), potassium chloride (KCl, ACS Reagent), sodium chloride (NaCl, 99.5%), sodium hydroxide, (NaOH, A.C.S. Reagent Grade), and hydrochloric acid (HCl, Certified A.C.S.). All the synthetic stomach / intestine reagents were purchased from Sigma-Aldrich (St. Louis, MO) except for the HCl which was purchased from Fisher Scientific.

The arsenic standards were prepared from arsenite [As(III)] and arsenate [As(V)] and purchased from SpexCertiPrep (Metuchen, NJ). All standards were verified against NIST 1640 (Gaithersburg, MD), Trace Elements in Water, based on total metal. All sample dilutions were prepared using 18 MΩ water (Millipore, Billerica, MA) on a Mettler (Columbus, OH) PM 1200 (0.001g) or a Mettler AG204 (0.0001g) balance. All off-line total metal measurements were determined using a single point method of standard addition. The QC for total metal measurements were in accordance with EPA Method 200.8.[3] The arsenic and iron concentrations in the synthetic stomach / intestine fluid were determined using the collision cell feature (As used He gas; Fe used H_2 gas) on the ICP-MS.

Drinking water distribution solids

The distribution solids were collected as a part of an earlier study.[4] The solids were collected as a hydrant flush material or as pipe scale from cross sections of used distribution pipes. The solids were allowed to air dry in a hood. The last solid in Table 1 (in Technical Note) was from a backwash of a filter in an iron removal plant. This solid should be representative of the arsenic iron oxide/hydroxides which have entered the distribution system because of inadequate filtration.[5-8] This solid was used to evaluate the synthetic stomach / intestine extraction because of its similarity to other distribution solids and because it was available in gram quantitities. The data for synthetic stomach / intestine extraction of this solid can be found in the ESI as Figure SI-4A and B and in Table 1 (in the Technical Note).

Automated continuous flow extraction system

Figure SI-1 contains a schematic of the continuous flow extraction system. In this system, the distribution solid sample is placed into the extraction cell and the remaining void volume of the extraction cell is filled with 250µM Teflon™ (Berghof/America Company, Coral Springs, FL). The extraction fluid is pumped into the extraction cell at a flow rate of 0.5mL/min. The void volume of the filled cell is approximately 100µL. This system and its operation have been previously described.[9] The only modification is the addition of a secondary external 0.45µm filter (Gelman, Ann Arbor, MI) which is located just after the extraction cell in the flow stream (see Figure SI-1). Included in the system is: a peri-pump (Rainin Dynamax, Emeryville, CA), an extraction cell (re-useable Peek™ guard column, Hamilton, Reno, NV), two automated six port valves (LabPRO Rheodyne Valve, Rohnert Park, CA), an Agilent 1100 Series Liquid Chromatograph (LC) (Palo Alto, CA) and an ICP-MS (Agilent 7500C, Palo Alto, CA). A couple of features of this system include: 1) the Agilent 1100 LC controls the entire system via electronic relays and a programmable series of timed events within the Agilent 1100 software; 2) the two automated six port valves and the LC switching valve facilitate chromatographic analysis (via injector #1) and flow injection analysis (via injector #2); 3) the LC autosampler has been reconfigured to allow two different standards to be injected; and 4) the LC autosampler injection loop volume is matched with both external injectors (valve #1 and #2; Figure SI-1) to allow for a single point standardization using the ICP-MS. The ICP-MS data associated with both chromatographic and total metal measurements are shown in the bottom left of Figure SI-1. Finally, it is worth noting that the extraction fluid is allowed to pass over the solid only once with this design.

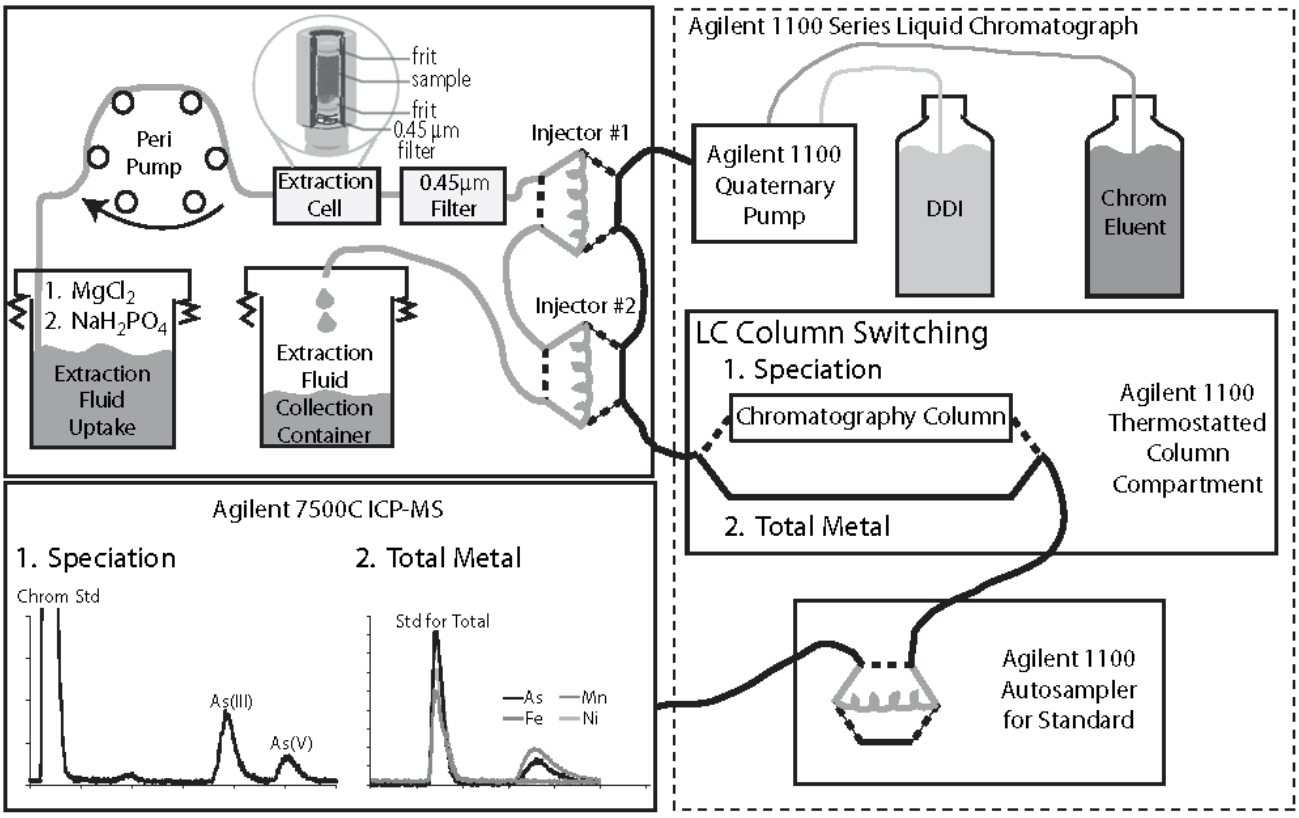


Fig. SI-1 Schematic of Continuous Flow Extraction System.

Estimating interconversion of As(III) and As(V) in the extraction fluids

The time between the extraction fluid (10mM $MgCl_2$ and 10mM NaH_2PO_4) coming into contact with the solid and the injection of the extract onto the column is less than two minutes (see flow path in Figure SI-1). During this time, the extracted As(III) could convert to As(V) and produce erroneous results. To assess the extent of the inter-conversion during this time interval, the extraction fluid was collected post extraction cell (approximately 20mL). The collected extraction solvent was split into three aliquots. The first aliquot was spiked with pure As(III) and held for one hr. The As(V) concentration was monitored in this aliquot using the off-line chromatographic analysis mode (see description below). The second aliquot was spiked with pure As(V) and held for approximately one hr. In this case, As(III) was monitored to estimate the conversion of As(V) to As(III). The third aliquot was unspiked and served as a control.

In order to estimate the extent of the oxidation/reduction that occurred during the one hr ammonium oxalate extraction period, a sample of the extract was taken and fortified in the manner just described. Stability studies were not conducted on the Ti citrate extraction because the dissolution occurs via a reductive mechanism.

Off-line extraction of distribution solids and chromatography separation used in stability studies

The 10mM $(NH_4)_2C_2O_4$ extraction was conducted by emptying the solids from the extraction cell into a 50mL centrifuge tube and extracting with 30mL of 10mM $(NH_4)_2C_2O_4$ for one hr on a shaker (Burrell Shaker, Pittsburg, PA). The shaker provided a gentle mixing of the solid without the use of a magnetic stir bar. The extraction cell and filter were also placed into the 50mL centrifuge tube to avoid sample losses. Additional one hr $(NH_4)_2C_2O_4$ extractions were completed until the extracted arsenic concentration became insignificant relative to the first extraction. The 50mL centrifuge tubes used for the $(NH_4)_2C_2O_4$ extraction were covered with aluminum foil.[1] After the $(NH_4)_2C_2O_4$, the extracts were rinsed once with 18 MΩ water prior to the Ti-Citrate extraction. A 30g aliquot of the Ti-Citrate extraction fluid (see safety note above) and a 3.5g of 1M $NaHCO_3$ were placed in the 50mL centrifuge tubes, the head space was purged with argon and the centrifuge tube was then placed on the shaker for two hrs. Only total arsenic concentrations were determined on these Ti-Citrate extracts. All off-line extraction fluids were centrifuged at 9000rpm (Model 5810R Eppendorf, Hamburg, Germany) for 10min prior to filtration through a 0.45µm (PTFE) syringe filter (Millipore, Bedford MA).

The off-line chromatographic system was used for all the samples collected to assess the interconversion of arsenic in the extraction solvent and for the analysis of the $(NH_4)_2C_2O_4$ extracts. This system has been described elsewhere,[10] and the chromatographic eluent and column were the same as used in the automated sequential extractor in Figure SI-1.

Enzymatic extraction of drinking water distribution solids

The synthetic stomach / intestine extraction approach was developed by Glahn et al.[11, 12] The limited supply of the drinking water solids made it necessary to scale the solid to synthetic stomach fluid ratio. The stomach fluid/solid used in Table 1 (in the Technical Note) (stomach fluid/solid = 160) is consistent with Rodriguez *et al.*[13] (stomach fluid/solid = 150) who utilized a synthetic stomach / intestine approach to estimate bioaccessibility of arsenic from soils, calcine and iron slag materials. The stomach extraction was carried out for one hr while the duration of the intestine extraction was two hrs. The samples were incubated in a temperature controlled room (37°C) on an orbital shaker (Labline Instruments, Melrose Park, IL). All samples were centrifuged at 9000rpm for 10min and then filtered through a 0.45µm filter (small volume < 2mL, PTFE ultrafree CL centrifuge filters, or large volume >2mL, PTFE syringe filters, Millipore, Bedford MA). The effect of an inert atmosphere was evaluated on a set of samples. In this case, all reagents were purged with argon for 15min and the head space of the centrifuge tube was purged prior to the incubation steps.

Table SI-1. Extraction Profile for Iron utilizing 10mM $MgCl_2$ at pH 8 as the Extraction Fluid[a]

Distribution Solid	ng/g of Fe in injection							
	Injection 1	Injection 2	Injection 3	Injection 4	Injection 5	Injection 6	Injection 7	Injection 8
1[b]	277	57	26	27	16	10	ND[c]	ND[c]
2	75	11	15	11	ND[c]	ND[c]	ND[c]	ND[c]
3	93	19	24	14	15	12	11	9
4	47	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]
5	677	52	44	20	24	ND[c]	ND[c]	ND[c]
6	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]

a. The iron concentration is based on a flow injection total metal measurement.
b. Injection number corresponds to the data presented in Figure 1 (in Technical Note) for Distribution Solid 1. All subsequent solids were analyzed in a similar manner.
c. The ND represents No Detect.

Table SI-1 summarizes the concentration of iron which is co-solubilized with the As by 10mM $MgCl_2$ for the six distribution solids in Table 1 (in the Technical Note). The data indicates that some iron is solubilized but the concentration drops off quickly and, in most cases, becomes indistinguishable from the background by the eighth injection.

Fig. SI-2 Extraction of As(III) / As(V) from Drinking Water Distribution Solid 1 using 10mM NaH_2PO_4 at pH 7.

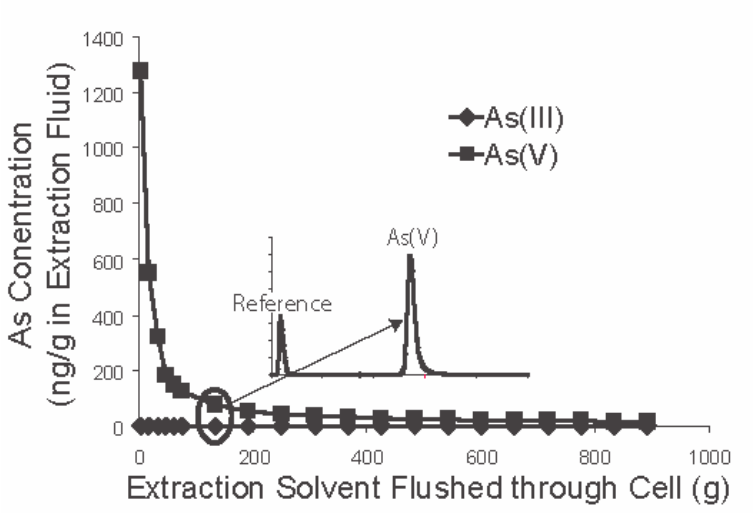


Figure SI-2, similar to Figure 1 (in Technical Note), is made up of individual chromatograms and the chromatogram associated with the second injection is displayed as an inset in Figure SI-2. This figure indicates that As(V) is liberated from the solid (initially 1300 ng As/g extraction fluid) by the 10mM NaH_2PO_4 and that a large percentage of the total arsenic is extracted by the first 100g of the extraction fluid that passes through the cell. The NaH_2PO_4 extraction profile also indicates that the concentration of As(III) is below the detection limit. The area under the 10mM NaH_2PO_4 extraction profile is 1380µg/g.

Fig. SI-3. Stability of As(V) and As(III) in 10mM $MgCl_2$ at pH 8

Fig. SI-3A. Stability of As(V) in 10mM $MgCl_2$ Extraction Fluid at pH 8

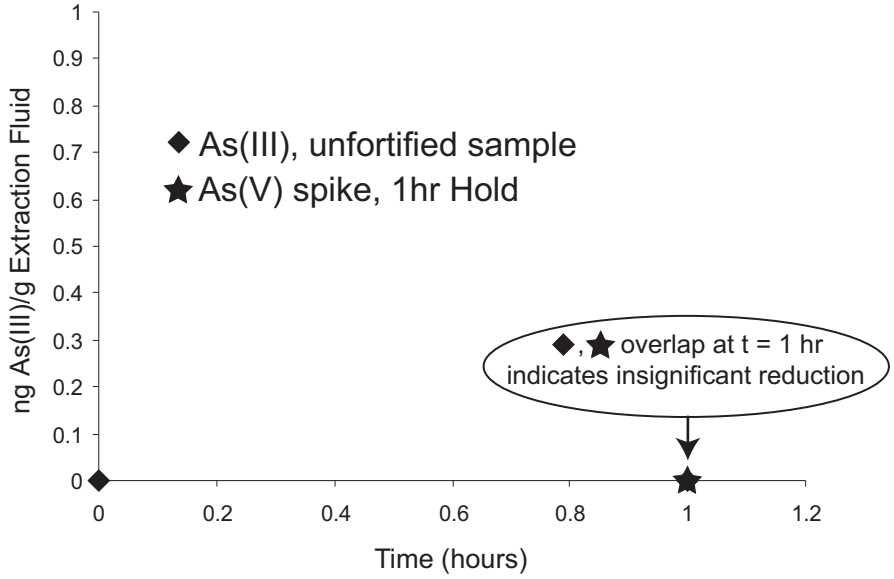


Fig. SI-3B. Stability of As(III) in 10mM $MgCl_2$ Extraction Fluid at pH 8

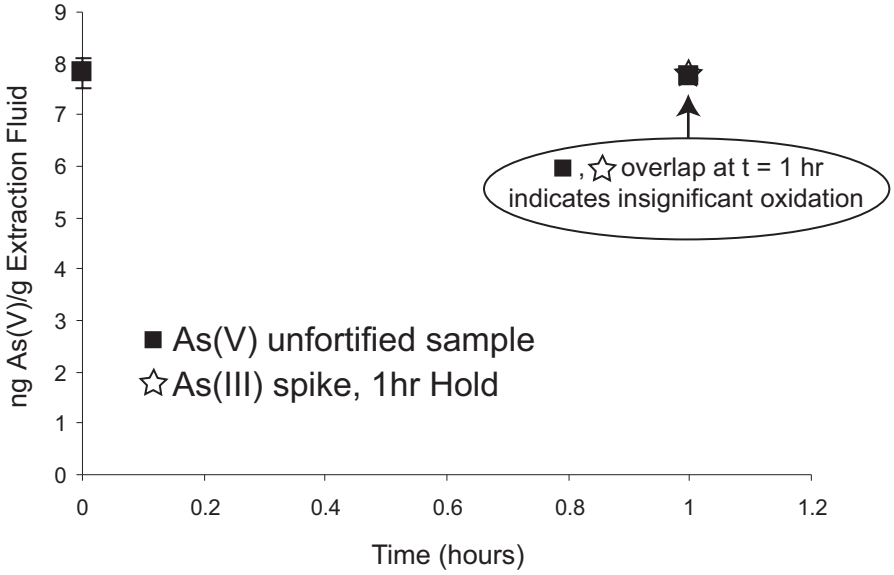


Figure SI-3A is a plot of As(III) concentrations vs. time for the unfortified and fortified samples. Figure SI-3B contains the corresponding As(V) data for the unfortified and fortified samples. The unfortified sample (♦ Fig. SI-3A; ■ Fig. SI-3B) was analyzed 3 times each at t = 0 and 1 hr. The analysis of the unfortified sample indicates that As(III) is below the detection limit (t=0 and t=1 hr; Fig. SI-3A) and the As(V) concentration is approximately 8ng/g (t=0 and t=1 hr; Fig. SI-3B). The unfortified sample indicates that the As(V) extracted from the media is not rapidly reduced to As(III). To evaluate the potential for rapid reduction, a sample fortified with As(V) was held for 1 hr and the As(III) concentration was still below the detection limit (★, Fig. SI-3A). Likewise, the rapid oxidation was evaluated by fortifying a sample with As(III). This sample was held for 1hr. The overlap of the unfortified (■, Fig. SI-3B) with the fortified (☆, Fig. SI-3B) sample at t=1 hr indicates that rapid oxidation did not occur. The variation of these measurements is graphically displayed via the 2 sigma error bars in Figure SI-3A and SI-3B. These error bars in most cases are smaller than the symbol shown in Figure SI-3. The data in Figure SI-3A and SI-3B clearly indicates that the species liberated from the solids is not rapidly oxidizing or reducing within the 2 minutes between the actual extraction and injection on the column. Similar stability data was collected for the other distribution solids using 10mM $MgCl_2$ and the data is summarized in Table 1 (in the Technical Note) in the columns labeled As(V) Stability and As(III) Stability.

Fig. SI-4. Sequential Extraction of Arsenic from Drinking Water Distribution Solids using a Synthetic Stomach and Intestinal Approach

The amount of stomach fluid to use in a physiological based extraction is an area of discussion in the literature.[13-16] Therefore, in Figure SI-4A, a wide range of stomach fluid volumes were evaluated in order to estimate the impact of this factor on the arsenic solubilized in the stomach/intestine. The solid (solid 7 in Table 1 in Technical Note) utilized in this evaluation was collected from the backwashed water from a filter in an iron removal drinking water facility. This solid, although not removed from the distribution system, was removed via filtration and should be characteristic of iron particulates that enter the distribution system because of inadequate filtration[5-8] and/or iron co-precipitation that occurs in the distribution system. However, it should be mentioned that this solid may not be the best surrogate for solids derived from distribution pipe scale.

The amount of arsenic liberated from the solid is estimated in both the synthetic stomach (Figure SI-4A) and intestine fluid (Figure SI-4B). The data were collected by weighing out four individual samples (~0.027g) of the solid and then repetitively extracting the sample with a fixed volume of synthetic stomach fluid. The arsenic liberated from the solid for each sequential extraction was then subjected to the intestine treatment. The amount of stomach fluid used in each extraction is represented by individual legends (■ = 2.1g, ▲ = 3.2g, ♦ = 5.3g and ● = 10.8g) in Figure SI-4 while the number of sequential extractions is displayed on the x-axis. The data in Figure SI-4A is reported as a concentration in the stomach fluid to facilitate the direct comparison to the arsenic remaining in the aqueous phase in the intestine (Figure SI-4B). To view the data in Figure SI-4A from an exposure assessment perspective, the volume of the extraction needs to be incorporated to estimate the total amount of arsenic liberated. The number of micrograms of arsenic released from the solid by the first stomach fluid extraction ranged from 37 μg (■ = 2.1g) to 80.7μg (● = 10.8g). The relative magnitude of these exposures can be placed in perspective by comparing it to the 20 μg exposure generated from drinking two liters of water per day at 10 ng/g (this concentration corresponds to the US drinking water Maximum Contaminant Level for arsenic).

The repetitive extraction of the solid with fresh synthetic stomach fluid was conducted for two reasons. The first reason was to estimate the exposure if the small amount of particulate was not able to be cleared from the stomach within a single ingestion event. Figure SI-4A clearly indicates that for small stomach fluid volumes (■ = 2.1g), the concentration for the second extract approximates the concentration associated for the first extract. The arsenic liberated by second - fourth sequential extractions drops off quickly. The percent drop is very dependent on extraction volume with a minimum (410%) associated with the smallest extraction volume and the largest percentage drop (6190%) is associated with the largest extraction volume. The second reason for investigating multiple extractions was to determine if all the arsenic could be liberated from the solid under these synthetic stomach fluid conditions. Additional sequential extractions (fifth - eighth, data not shown) were conducted with the 10.8g (●) extraction volume and the summation of the amount of arsenic recovered from extraction one - eight account for 85% of the total digest value for this solid. This mass balance experiment also indicated that 64% (of the total digest concentration) is liberated by the first extraction for the 10.8g (●) volume.

Figure SI-4B utilizes a similar format to Figure SI-4A but reports the percentage of arsenic that remains in the aqueous phase after the intestinal treatment. Figure SI-4B indicates that less than 1% of the arsenic solubilized by the first stomach extraction remains in the aqueous phase after the intestinal treatment. This observation is independent of the volume of stomach fluid utilized in Figure SI-4A. In each case, the intestinal treatment resulted in the visual formation of a red/orange precipitate resembling the iron oxide/hydroxide precipitates usually associated with iron co-precipitation. A likely explanation for the precipitation and loss of arsenic from the aqueous phase is related to the fact that the iron solid formed in the drinking water treatment plant is predominantly ferric iron. The ferric iron, which has co-absorbed arsenic, enters the stomach where the solubility of ferric iron is dramatically increased by the relatively low pHs associated with the stomach. This low pH solution then enters the intestine where the pH begins to rise. The solubility of the ferric ion begins to decrease with the rising pH and thus, the intestine environment mimics the co-precipitation of arsenic with iron used in drinking water treatment plants. This notion that the arsenic is removed during the intestine treatment via a ferric ion precipitation is further supported by the observation that the iron precipitation reaction occurs even when the extraction is conducted in an inert atmosphere with all extracting solutions purged with argon to minimize dissolved oxygen effects. The inert atmosphere experiment should greatly reduce the potential for oxidizing ferrous ion to ferric ion and thereby, minimize the formation of a precipitate via this pathway.

The second through fourth extraction indicate an upward trend in the percentage of arsenic that remains in solution in the intestine fluid except for the 2.1g extraction. This contradiction to the trend may be explained by the fact the volume associated with the 2.1g extraction is sufficiently small to allow the co-solubilized iron concentration to be high enough (relative to the large extraction volumes) to facilitate the co-precipitation when the pH is raised. This notion is also supported by the fact that by the fourth extraction cycle, the larger volume extracts have ceased to produce a precipitate when the intestinal treatment is applied.

Figure SI-4A. Sequential Extraction of Arsenic from Drinking Water Solids using Various Amounts of Synthetic Stomach Fluid

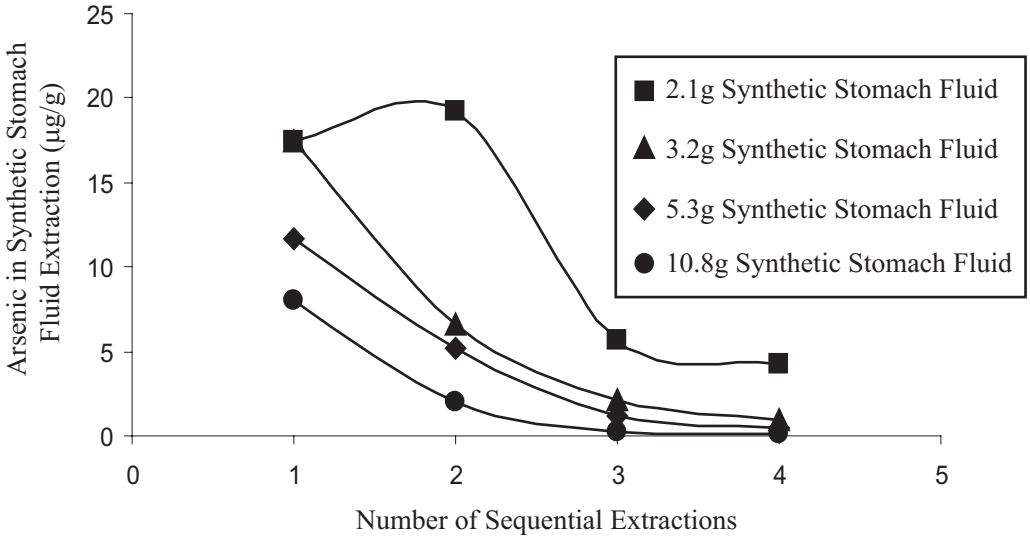
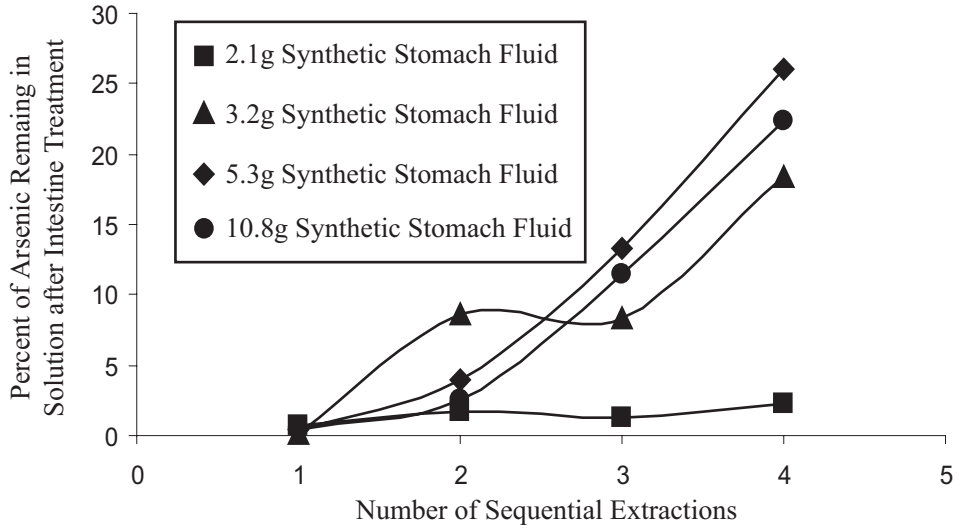


Figure SI-4B. Percentage of Arsenic Remaining in Solution after Multiple Sequential Stomach/Intestine Extractions



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