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Accelerated Analyte Uptake on Single Beads in Microliter-Scale Batch Separations Using Acoustic Streaming: Plutonium Uptake by Anion Exchange for Analysis by Mass Spectrometry

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The use of acoustic streaming as a noncontact mixing platform to accelerate mass-transport-limited diffusion processes in small-volume heterogeneous reactions has been investigated. Single-bead anion exchange of plutonium at nanomolar and subpicomolar concentrations in 20 μL liquid volumes was used to demonstrate the effect of acoustic mixing. Pu uptake rates on individual $\sim 760\text{ }\mu\text{m}$ diameter AG 1 \times 4 anion-exchange resin beads were determined using acoustic mixing and compared with Pu uptake rates achieved by static diffusion alone. An 82 MHz surface acoustic wave (SAW) device was placed in contact with the underside of a 384-well microplate containing flat-bottomed semiconical wells. Acoustic energy was coupled into the solution in the well, inducing acoustic streaming. Pu uptake rates were determined by the plutonium remaining in solution after specific elapsed time intervals using liquid scintillation counting (LSC) for nanomolar concentrations and thermal ionization mass spectrometry (TIMS) analysis for the subpicomolar concentration experiments. It was found that this small batch uptake reaction could be accelerated by a factor of about 5-fold or more, depending on the acoustic power applied.

A continuing trend in chemistry is to conduct processes in or on smaller and smaller volumes or surfaces. This trend is seen throughout all areas of analytical chemistry and is evident with respect to surface-confined chemical reactions and in the assembly of molecules and nanostructures. However, small fluid volumes and small surface areas present significant transport problems. In this paper, we are specifically concerned with the uptake of actinide ions, particularly plutonium, on single anion-exchange resin beads in microliter volume batch reactions. These reactions are normally very slow due to diffusion limitations in stationary fluids. The noncontact method of mixing solutions at microliter scales using acoustic streaming, to be described below for small volume batch ion exchange, is also more generally applicable to a variety of small-scale analytical processes where mass transport is an issue.

For example, at volumes $\ll 1\text{ mL}$, mechanical agitation becomes inefficient due to the more pronounced effects of viscosity

characteristic of low Reynolds number systems.¹ Moreover, laminar flow processes in microchannels at low Reynolds numbers limit mixing between adjacent fluids. In addition, sorptive processes at interfaces, from a bulk solution to the surface, can become limited by diffusion through a region of depleted concentration, i.e., the diffusion layer, if the solution is not mixed. Flow approaches for working with small volumes, especially using electroosmotic rather than pressure-driven flow, have proliferated in the field of chip-scale microfluidics. Far less work, however, has been done on small-volume batch methodology. We are also concerned with limiting possible contamination of small-volume samples with surface-active molecules or with species that will interfere with accurate trace analysis. Therefore, even if miniature, mixing methods where a device contacts the sample, be it a mechanical mixer or an ultrasonic horn, are undesirable.

Acoustic methods are finding increased uses in analytical chemistry. Aside from the well-known use of acoustic wave devices for gas-phase and liquid-phase sensing,² acoustic waves are also finding increasing use for manipulating particles and fluids on small scales. The use of acoustic fields, the radiation force, and acoustic streaming for biosensors and bead-based assays has been reviewed recently.^{3,4} Acoustic forces have been used to manipulate droplets and to perform assay operations on surfaces.^{5–9} Acoustic methods have also been developed for pumping fluids in small channels.^{10–16} Acoustic streaming has been noted as a method to

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promote liquid mixing in closed cylinders,^{17,18} in the wells of microtiter plates,⁹ within fluids in microchannels,^{2,15,16,19–21} and in single liquid droplets.^{7,19} The use of acoustic streaming to promote transport and mixing has been demonstrated to accelerate the rate of an immunochemical reaction on a planar sensor surface,²² to accelerate the rate of an electrochemical reaction at a planar electrode,²³ and to accelerate equilibrium in the adsorption of phenol on the surfaces of activated carbon powders or porous polystyrene beads.²⁴ Both cavitation microstreaming using an air bubble^{25,26} and acoustic streaming induced by surface acoustic wave (SAW) devices have been used to accelerate DNA hybridization and dye labeling on microarrays.^{9,27} Of particular interest is the possibility of coupling acoustic waves generated outside a small rigid container or chip into the fluid within to promote mixing. Such noncontact approaches, using small planar transducers to generate the acoustic waves, could accelerate small-volume batch processes that are currently rather slow due to diffusional limitations in static fluids.

Ion exchange represents a mass-transport-controlled process of considerable use in analytical chemistry for collecting and concentrating trace elements for analysis. In small batch scales, it can be used to collect ionic analytes on single resin beads. For example, in the field of environmental analysis, single-bead anion exchange in microliter volumes can be used as a microchemical purification step for actinides and other trace elements.^{28–31} Similarly, uptake on single resin beads is routinely used to prepare ion sources for thermal ionization mass spectrometry (TIMS).^{29,30,32–38} However,

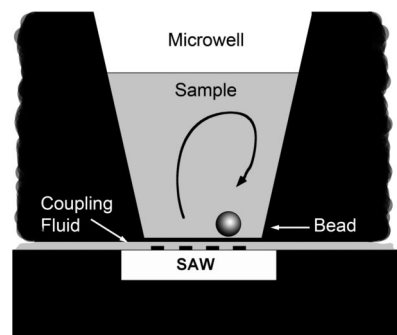


Figure 1. Cross-sectional schematic diagram of a single-bead uptake experiment with mixing provided by coupling acoustic energy from a SAW device into the container through the bottom, illustrated with a microwell. Not drawn to scale.

these uptake processes require many hours to come to equilibrium in static unmixed liquid volumes.

Performing separations on decreasing volume scales becomes essential as the desired detection limits decrease, since the reagents used contain contaminants. Even with high-purity reagents, any chemical separation procedure will introduce new impurities into the sample during its chemical purification. If the introduction of the new impurities is of significance, then the only way to minimize them is to scale down the separation procedure. Background interferences in TIMS are often limiting factors that determine the achievable detection limits. These interferences originate primarily from the ion source and can only be reduced by preparing cleaner sources from more highly purified analyte.

In this article, we investigated the use of acoustic streaming to accelerate single-bead anion-exchange uptake of plutonium at nanomolar and subpicomolar concentrations in 20 μL liquid volumes, which can serve as a prototypical example of a batch, small-volume, single-bead, uptake process. Tetravalent plutonium forms a dianionic hexanitrate complex, $\text{Pu}(\text{NO}_3)_6^{2-}$, in strong nitric acid solutions. Since few cations of alkali, alkaline earth, or transition metal elements will form negatively charged nitrate complexes, anion exchange can be used to selectively capture anthropogenic actinides from environmental samples.^{38,39}

We examined Pu uptake rates on an $\sim 760\text{ }\mu\text{m}$ diameter AG 1 $\times 4$ anion-exchange resin bead by static diffusion alone and with acoustic mixing. The sample container, a flat-bottomed semiconical microplate well, was placed on an 82 MHz SAW device as shown in Figure 1. The SAW device generated acoustic waves that coupled into the sample in the microwell to create mixing fluid flow by an acoustic streaming mechanism. We analyzed the plutonium remaining in solution after specific elapsed time intervals to determine uptake rates, using liquid scintillation counting (LSC) for the nanomolar concentrations and TIMS analysis for the subpicomolar concentration experiments. We found that this small batch uptake reaction could be accelerated by a factor of about 5-fold or more, depending on the acoustic power applied. As a result, a small-volume single-bead uptake reaction can approach equilibrium in a few hours instead of requiring overnight (or longer) contact times.

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THEORETICAL SECTION

Ion exchange in beads is recognized to be a diffusional phenomenon limited by either the diffusion of ions within the bead—"particle diffusion"—or diffusion of ions in a film or "diffusion layer" at the bead/solution interface—"film diffusion".^{40,41} The diffusion layer is an idealization of the flow and concentration gradients from surface to bulk solution, defining a fictitious liquid layer of defined thickness without any convection through which species must diffuse from the bulk solution to the surface. Though not a physical quantity that can be measured directly, the concept of the diffusion layer has proven to be extremely useful for modeling diffusion kinetics at solid interfaces.⁴¹

The 20 μL sample solutions in our experiments using LSC measurements contain on the order of 2×10^{-13} mol of Pu at 11 nM concentration. Experiments using TIMS measurements were conducted at over 4 orders of magnitude lower concentration (0.5 pM). Film diffusion is known to predominate in ion-exchange processes at low concentrations.⁴¹ In addition, based on a capacity of 1 mol equiv of ion-exchange groups per liter of resin, and a bead volume of 2×10^{-7} L for our 760 μm diameter beads, we can estimate that there are approximately 2×10^{-7} mol of exchange groups per resin bead. Thus, the bead capacity vastly exceeds the amount of Pu in our experiments at both concentration levels.

It can be further estimated that the volume of resin material required to sorb all of the Pu in a 20 μL sample in the LSC experiments is found within less than 3 Å of the resin bead surface or less than the diameter of one of the phenyl groups of the polymer backbone. Since film diffusion, not particle diffusion, is rate limiting, Pu captured at the bead surface is not expected to accumulate there. Nevertheless it is instructive to consider that the outer 1 μm shell of the 760 μm diameter resin bead contains sufficient ion-exchange equivalents to absorb 10^{-9} mol of dianionic Pu complex, compared to the 10^{-13} mol of Pu in our highest concentration experiments.

Ion-exchange processes are most easily modeled considering the ideal case of isotopic exchange (e.g., exchanging $^{14}\text{NO}_3^-$ with $^{15}\text{NO}_3^-$) where the system is at chemical equilibrium (except for the isotopic distribution) the entire time. In this case, given film diffusion control and a liberal excess of ion-exchange material, Helfferich gives eq 1 to describe the time, t , within which the concentration of an analyte species is reduced to a fraction X of its original concentration in a batch operation with a finite solution volume.

$$t = -\left(\frac{r_0 V \delta}{3 \bar{V} D}\right) \ln(X) \quad (1)$$

Here, X is the ratio of analyte concentration at time t (in seconds) to the initial analyte concentration, \bar{V} and V are the volumes of the ion-exchange media and the sample solution, respectively, r_0 is the average radius of the ion-exchange resin bead, D is the diffusion coefficient of the analyte, and δ is the Nernst diffusion layer thickness. This is easily rearranged to a first-order rate law form as in eq 2, where the rate constant, k , is equivalent to the quantity $3\bar{V}D/r_0V\delta$.

$$\ln(X) = -t\left(\frac{3\bar{V}D}{r_0V\delta}\right) = -kt \quad (2)$$

Thus, the rate constant can be determined from the slope of $-\ln(X)$ plotted against time. When all parameters comprising k are known, the observed rate constant k_{observed} can be directly compared to the expected rate constant. Alternatively, the diffusion layer thickness can be calculated from k_{observed} and values for the other parameters. Although eq 2 is strictly valid for pure isotopic exchange between a solution and an ion-exchange resin, it can be applied to the exchange of trace components.⁴² In our experiments we are exchanging divalent $\text{Pu(IV)(NO}_3)_6^{2-}$ complexes for NO_3^- anions, the composition is not at equilibrium, and the concentration of the analyte in solution declines as the system moves toward equilibrium.

The maximum sorption of an analyte is limited by its equilibrium distribution coefficient, K_D , which can be defined in terms of the moles of analyte in the bead, n_{bead} , and in solution, n_{soln} :

$$K_D = \frac{n_{\text{bead}}/\bar{V}}{n_{\text{soln}}/V} \quad (3)$$

For $\text{Pu(NO}_3)_6^{2-}$, the distribution coefficient on a strongly basic anion-exchange resin is 1×10^4 8 M HNO_3 .^{43,44} Using 760 μm diameter ion-exchange resin beads and 20 μL of sample solution for these experiments, (see the Experimental Section), we cannot expect to adsorb more than 99% of the Pu into the resin bead, regardless of how long we allow the system to equilibrate.

Most parameters comprising eq 1 can be measured directly. However, both the diffusion coefficient and the diffusion layer thickness are dependent on quantities that may vary over the range of experiments to be described. Specifically, the diffusion coefficient for a given species is sensitive to changes in temperature and viscosity according to the Stokes–Einstein equation:

$$D = \frac{kT}{6\pi a \eta} \quad (4)$$

where kT is the thermal energy in joules and a is the hydrodynamic radius of the solute in a solution of viscosity η .

The magnitude of the diffusion layer thickness depends on the velocity of the solution relative to the surface. For ion-exchange columns, the diffusion layer thickness may be estimated by the empirical relationship^{41,45}

$$\delta = \frac{0.2r_0}{(1 + 70r_0v)} \quad (5)$$

where r_0 is the bead radius (in cm) and v is the linear flow velocity (in cm/s) of the solution. Generally, the diffusion layer thickness is estimated to be of the order of 10–100 μm .

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For isothermal experiments, the parameters \bar{V} , V , r_0 , and D in the rate constant do not change. Therefore, changes in the observed rate, k_{observed} , as a result of mixing are ascribed to changes in the thickness, δ , of the idealized diffusion layer.

EXPERIMENTAL SECTION

Chemicals. Optima grade concentrated nitric and concentrated hydrochloric acids (Fisher Scientific) were used as received for ^{244}Pu experiments (TIMS), whereas Trace Metal grade concentrated nitric acid (Fisher Scientific) was used as received for ^{238}Pu experiments (LSC). All solutions were prepared using deionized water (Barnstead Nanopure Diamond, $18.2\text{ M}\Omega\cdot\text{cm}$ and Barnstead E-Pure, $18.3\text{ M}\Omega\cdot\text{cm}$ for TIMS and LSC experiments, respectively). Solid amaranth (CAS no. 915-67-3; Pharmaceutical Laboratories, C.I. 184) and methyl green (CAS no. 7114-03-6; Pharmaceutical Laboratories, C.I. 685) dyes were each diluted with deionized water to $5.5 \times 10^{-5}\text{ M}$ and $5.3 \times 10^{-5}\text{ M}$, respectively. Plutonium isotope concentrations were 1670 Bq/mL ^{238}Pu in 7.5 M HNO_3 for the LSC experiments, which is $2630\text{ fg}/\mu\text{L}$ or 11 nM . For TIMS experiments the concentrations were $0.1\text{ fg}/\mu\text{L}$ ^{239}Pu and nominally $0.12\text{ fg}/\mu\text{L}$ ^{244}Pu in 2 M HCl .

Ion-Exchange Resin Beads. We used 20–50 mesh AG 1 \times 4 anion-exchange beads from Bio-Rad Laboratories, Hercules, CA. From the original stock, we attempted to select beads of a consistent size, typically $\sim 760\text{ }\mu\text{m}$ in diameter. Consistent anion-exchange behavior required careful bead pretreatment. Prior to use, beads were bathed in (1) 9 M HCl for at least 15 min (3 \times), (2) 1.2 M HCl for 15 min (3 \times), and (3) deionized water for 15 min (3 \times). Subsequently, the beads were converted to the nitrate form by soaking in 7.5 M nitric acid for at least 30 min (3 \times) and were then liberally rinsed with deionized water.

Acoustic Mixing. SAW devices used to couple acoustic waves into the reaction volume and induce mixing by acoustic streaming were obtained as SAW Station Three systems from Advantix AG, Germany. Each system consisted of a single mounted 82 MHz SAW device and an adjustable radio frequency power supply which we operated at power settings between 20 and 35 dBm (0.1 – 3.2 W). All dye uptake experiments were performed in 5 mL polystyrene cups, whereas all Pu sorption experiments were performed in 384-well microplates, (Greiner Bio-One, Kremsmünster, Austria, part no. 788096) with flat-bottomed $25\text{ }\mu\text{L}$ volume semiconical wells. The plates were made of cyclic olefin copolymer (COC). Water was used as a coupling fluid between the SAW device and the reaction container. Parafilm was used to seal the top of the microwells to prevent evaporative losses during experiments. While static diffusion experiments could be run in parallel, only one sample at a time could be mixed using the SAW station, requiring that samples mixed by acoustic streaming experiments be processed serially. The effect of applied SAW power on sample temperature was monitored after 30 min of continuous mixing with a digital thermometer (OE871, Omega Engineering, Stamford, CT). The samples evaluated in these studies consisted of $20\text{ }\mu\text{L}$ of deionized water.

Dye Uptake Experiments. Two AG 1×4 resin beads of approximately the same size (wet diameter $\sim 760\text{ }\mu\text{m}$) were each placed into separate 5 mL disposable polystyrene containers. After the resin beads had dried completely, $100\text{ }\mu\text{L}$ of a dilute amaranth solution was deposited in the center of each container and the resin beads were centered in the liquid droplets. One of the

samples was then immediately placed on the SAW station, while the second sample was placed on a static benchtop. We observed dye uptake in each of the beads over a period of 1 h by optical microscopy (Olympus SZX12 reflectance microscope) and captured digital optical images (Olympus C-3030 digital camera) at several stages of the dye uptake process. As a control experiment, we also prepared a third sample consisting of a resin bead and $100\text{ }\mu\text{L}$ of solution containing cationic methyl green in a polystyrene container and monitored its progress for up to 20 min .

Pu Uptake Using Liquid Scintillation Counting. The determination of Pu uptake rates by LSC utilized a Perkin-Elmer Tri-Carb 3100 liquid scintillation analyzer (Waltham, MA) and the ^{238}Pu isotope, which is a pure α emitter with a short half-life enabling practical radiometric detection. Each data point was determined by contacting an anion-exchange bead with $20\text{ }\mu\text{L}$ of a 1670 Bq/mL ^{238}Pu solution in 7.5 M HNO_3 . This solution contained 52.6 pg of ^{238}Pu at 11 nM concentration and provided 33.3 Bq per experiment. Immediately following the contact experiment (at a specific elapsed contact time), $15\text{ }\mu\text{L}$ of the original $20\text{ }\mu\text{L}$ solution was sampled from the microwell and transferred to a plastic liquid scintillation vial which had been prefilled with 15 mL of Ultima Gold scintillation cocktail (Perkin-Elmer Life and Analytical Sciences, Inc., Waltham, MA).

The scintillation method detects all radioactive species in solution, including species such as Pu(III) that could be in the ^{238}Pu standard that do not form anionic complexes that exchange into the resin bead. These spectator ions bias the use of a scintillation measurement to determine the Pu(IV) remaining in the liquid phase. We observed that with or without acoustic streaming, the apparent Pu uptake at extended contact times reached a plateau of 89% , 10% below the theoretically calculated value of 99% uptake. In separate experiments on an identically prepared standard, using an anion-exchange column separation, we confirmed the presence of radioactive species that were not retained on the column from strong nitric acid, most likely Pu(III). The Pu uptake data determined by scintillation counting were corrected by determining a normalization factor ($99/89$) to bring the final values to 99% uptake.

Control experiments adding ^{238}Pu standard to empty microwell plates were performed to ensure that the surface of the microwell plate had no affinity for Pu. These controls were exposed to the same conditions as in the bead-contact experiments. The final solution activities were in excellent agreement with the ^{238}Pu stock standard, thus assuring that the uptake data for ^{238}Pu in the microwell experiments were exclusively due to the anion-exchange bead.

Pu Uptake Using Thermal Ionization Mass Spectrometry. Diffusion experiments at subpicomolar Pu levels were monitored using TIMS to analyze the Pu remaining in the sample liquid after specific contact times. Anion-exchange beads were exposed to nominally 2.4 fg of ^{244}Pu in $20\text{ }\mu\text{L}$ of 7.5 M HNO_3 , corresponding to a concentration of 0.5 pM , for specific periods of time, t . The liquid samples, containing ^{244}Pu that had not been captured by the anion-exchange bead, were recovered and then spiked with 2 fg of ^{239}Pu . TIMS analysis, which determines only Pu(IV) and not Pu(III) because of the anion-exchange resin bead method of source preparation, was used to determine the ratio of ^{244}Pu (IV) to ^{239}Pu (IV), i.e., $(^{244}\text{Pu}(\text{IV})/^{239}\text{Pu}(\text{IV}))_{\text{time}=t}$. Similarly, this ratio

was determined for samples that were not exposed to an anion-exchange bead, representing time = 0, that were spiked with the same amount of ^{239}Pu . Then, the ratio of values at times t and 0 ($^{244}\text{Pu}(\text{IV})/^{239}\text{Pu}(\text{IV})_{\text{time}=t}/(^{244}\text{Pu}(\text{IV})/^{239}\text{Pu}(\text{IV}))_{\text{time}=0}$) gives the fraction of $^{244}\text{Pu}(\text{IV})$ left in solution at time t .

The procedures were carried out as follows. Samples containing nominally 2.4 fg of ^{244}Pu in 2 M HCl were prepared on Teflon pads. These samples were transposed to HNO_3 by evaporating the solution on the pads under a heat lamp and subsequently adding 7.5 M HNO_3 so that the final sample volume at this stage was 10 μL . For bead uptake by static diffusion or acoustic streaming, each 10 μL sample was transferred from its Teflon pad to a microwell containing one resin bead. Next, each Teflon pad was rinsed with 10 μL of 7.5 M HNO_3 and the rinse was added to the microwell of the respective sample (for a total sample volume of 20 μL in each well). Then the microwells were covered with Parafilm to prevent evaporation of the sample fluid, and for each bead experiment, diffusion was allowed to progress for a specific period of time. Following each sample/bead contact, the 20 μL volume from the well was transferred back to the original Teflon pad. Next, the microwell was rinsed with 20 μL of 7.5 M HNO_3 , which was then added to its respective sample on the Teflon pad. Then we added 2 fg of ^{239}Pu in 2 M HCl to each sample and again transposed each sample back into 7.5 M HNO_3 solution. These samples were then prepared for analysis by TIMS using the procedure described previously.³⁴ Briefly, the Pu(IV) in the sample in nitric acid is exchanged into a single smaller anion-exchange bead which is used to prepare the ion source on the TIMS filament. Because this source preparation method does not capture Pu(III), this analysis approach only determines Pu(IV) and not any Pu(III) that may have been a spectator ion in the sample during the anion-exchange uptake experiment. This holds for both the bead-contact samples and the baseline isotopic measurements, representing time = 0, described next.

Samples containing nominally 2.4 fg of ^{244}Pu in 2 M HCl were prepared on Teflon pads and transposed to 7.5 M HNO_3 as described above. Then 2.0 fg of ^{239}Pu in 2 M HCl was added to each of these samples and again transposed to 7.5 M HNO_3 . We prepared 11 such samples and captured the Pu(IV) on a small anion-exchange bead to determine an average value of 1.33(3) for the $^{244}\text{Pu}(\text{IV})/^{239}\text{Pu}(\text{IV})$ isotopic ratio at time = 0. Because the amount of ^{239}Pu was constant for all samples, the ratio of $^{244}\text{Pu}(\text{IV})/^{239}\text{Pu}(\text{IV})$ at time t to $^{244}\text{Pu}(\text{IV})/^{239}\text{Pu}(\text{IV})$ at time 0 yields the fraction of $^{244}\text{Pu}(\text{IV})$ in solution at time t , with the remainder of the ^{244}Pu being adsorbed into the anion-exchange resin bead according to mass balance.

RESULTS AND DISCUSSION

Colorimetric Observation. Our first experiments were designed to qualitatively investigate whether or not acoustic mixing could be used to accelerate the equilibration between a given solution and an ion-exchange resin bead. The polystyrene/divinylbenzene cross-linked copolymer beads used in our experiments contained positively charged quaternary amine functional groups with an affinity for anions. We monitored the ion exchange between a resin bead and a dilute solution of a trivalent strong anionic dye, amaranth. Although the solution color is very pale, concentration of the colored dye molecules on the bead is readily

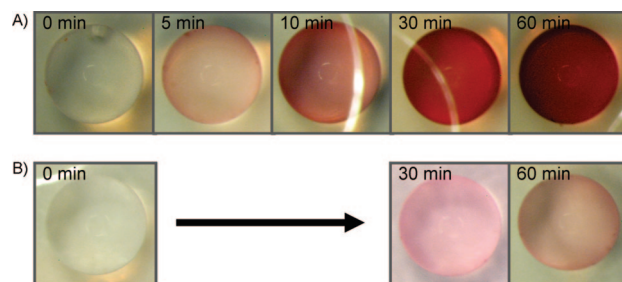


Figure 2. Optical microscopy images comparing the sorption of a strong trianionic dye, amaranth (5.5×10^{-5} M), by ~ 760 μm strong-anion-exchange resin beads over a 1 h time period with acoustic mixing at 25 dBm (A) and by diffusion alone (B).

observed. Figure 2 compares the uptake of the colored dye onto resin beads with acoustic streaming at 25 dBm and without streaming over a period of 1 h. After 10 min, the bead with mixing is clearly turning red, while the bead without mixing is quite pale even after 60 min. The bead with mixing is intensely red after 60 min. Although not quantitative, we estimated from the color changes observed that the ion-exchange process with acoustic mixing was $\sim 5\times$ faster than diffusion alone. We were not able to observe any color change in a bead in contact with a dilute solution of methyl green, a divalent cationic dye, after streaming at a higher power setting (30 dBm or 1 W) for 20 min. This control experiment confirms that ion-exchange processes are involved.

Pu Uptake as Analyzed by Liquid Scintillation Counting.

Pu uptake experiments were carried out in flat-bottomed semi-conical wells of 384-well microplates. With the use of suspensions of small hydrogel beads to indicate fluid movement, it was visually confirmed that mixing was induced by turning the SAW device on at settings such as 20 or 25 dBm and ceased immediately on turning the power off. Furthermore, in our experiments, the anion-exchange beads in our 20 μL samples were visibly perturbed when the acoustic power was turned on at 25 dBm (or higher), indicating bulk movement of the fluid relative to the beads.

The extent of Pu uptake by single anion-exchange beads after specific contact times, using a separate bead for each contact time data point, was determined by sampling and analyzing 15 μL of the 20 μL liquid phase. Using a Pu isotope standard with a relatively high specific activity, we could directly measure the remaining activity in a sample of the liquid using LSC as described in the Experimental Section. The initial 20 μL sample solution contained 52.6 pg of ^{238}Pu at 11 nM concentration, and control experiments verified that the loss of Pu from solution was due to uptake on the anion-exchange bead and not due to contact with the walls of the microwell plate. Uptake data were corrected for scintillation due to isotopes that were not Pu(IV) and hence do not participate in the anion-exchange chemistry, as described in the Experimental Section. For each elapsed time, multiple experiments were carried out and averaged.

Figure 3 shows the uptake of Pu as a function of time, comparing experiments where static diffusion is the only transport mechanism with experiments using acoustic streaming to mix the solution. The SAW device was operated at a power setting of 25 dBm (0.32 W) which does not significantly raise the solution temperature. The plot shows data collected over two experimental campaigns, indicated by circles and squares, which were in good agreement. (The data were pooled for subsequent analysis.) From

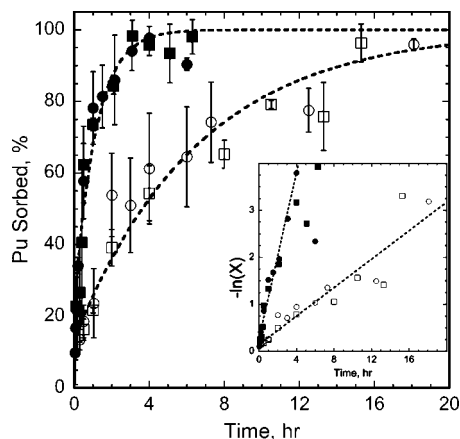


Figure 3. Percentage of $^{238}\text{Pu}(\text{IV})$ sorbed from solution as a function of time with (●, ■) and without (○, □) acoustic streaming at 25 dBm. Sorption of $^{238}\text{Pu}(\text{IV})$ was determined by LSC normalizing the data as described in the Experimental Section. Each data point represents an average of 2–5 replicate measurements. Dashed lines indicate trendlines estimated from eq 1 after obtaining a fit of the data in the inset. Inset: Plot of the negative natural logarithm of the fraction, X , of ^{238}Pu remaining in solution vs time with and without acoustic streaming. Dashed lines indicate linear regression of data up to 80% uptake.

this figure, it is clear that acoustic mixing significantly accelerates the uptake of Pu. As a rough estimate of the initial acceleration due to mixing, we found that rate constants calculated from 30 min elapsed time data points indicate approximately 5 times higher rate with mixing compared to static diffusion.

The inset in Figure 3 shows these data plotted as $-\ln(X)$ according to eq 2. The experimental data for this observed anion-exchange process are not strictly linear. Nevertheless, for comparison purposes, we calculated the average rate constants with and without streaming for the first 4 and 18 h for the mixed and unmixed cases, respectively (corresponding to >80% sorption). Linear regression gave observed rate constants of $2.5(2) \times 10^{-4} \text{ s}^{-1}$ with streaming and $0.42(4) \times 10^{-4} \text{ s}^{-1}$ without (R^2 values of 0.93 and 0.87, respectively), indicating a 5.8-fold increase in uptake rate by using the acoustic mixer, an increase consistent with that based on visual inspection of the trivalent anionic dye taken up by the beads (Figure 2). Rate data comparisons obtained by this approach are summarized in Table 1.⁴⁶

In separate acoustic streaming experiments, the Pu fraction sorbed after 30 min was determined at several power settings. Results are shown in Table 2 along with additional observations and relevant parameters. At 25 dBm, the initial rate at 30 min is 5.7 times greater than with 0 dBm (static diffusion), in agreement with the numbers noted above. Sorption rates rise at power settings above 25 dBm, with a maximum initial uptake rate 9.6 times higher than the rate due to static diffusion alone observed at 28 and 31 dBm.

Figure 4 and Table 2 indicate the heating of the sample solutions as a function of dBm setting. At 25 dBm and below,

Table 1. Observed Ion-Exchange Rate Constants for Pu Uptake Calculated by Linear Regression of Data from Figures 3 and 5

experiment	$k_{\text{observed}} (\text{s}^{-1})^a$	times faster than static diffusion	N^b	R^2
LSC–diffusion ^c	$4.2(4) \times 10^{-5}$	1.0	9	0.87
LSC–streaming ^d	$2.5(2) \times 10^{-4}$	5.8 ^e	8	0.93
TIMS–diffusion ^c	$5.9(5) \times 10^{-5}$	1.0	7	0.96
TIMS–streaming ^d	$1.8(1) \times 10^{-4}$	3.1 ^f	7	0.97

^a Brackets denote standard deviation. ^b For LSC measurements, N is the number of elapsed time points, each of which is the average of 2–5 measurements. ^c Average over 18 h (>80% sorption). ^d Average over 4 h (>80% sorption) at 25 dBm. ^e Calculated with respect to k_{observed} for static diffusion as determined by LSC. ^f Calculated with respect to k_{observed} for static diffusion as determined by TIMS.

solution temperatures and viscosities are relatively constant; however, at higher settings the solution is heated significantly. Temperature and related changes in viscosity directly affect the magnitude of diffusion coefficients according to eq 4. An increase in temperature, instead of more vigorous mixing, might then be offered as an explanation for the accelerated sorption kinetics at higher dBm settings. However, the viscosity of the 7.5 M HNO_3 solution used in our experiments actually *increases* with increasing temperature.⁴⁷ The effects of increased solution temperature are offset by the viscosity increase, such that the predicted diffusion coefficient (eq 2) of a solute over the range of SAW power settings (0–31 dBm) changes less than 3%. Therefore, the increased uptake rate at higher power settings is most likely due to more vigorous mixing.

We used the observed rate constants to estimate the apparent diffusion layer thicknesses, δ_{observed} (Table 2), at different power settings according to $\delta = 3\sqrt{D/r_0}V/k_{\text{observed}}$. We estimated that the diffusion layer thickness was 153 μm without streaming. In contrast, the estimated diffusion layer thickness with acoustic streaming was as small as 16 μm at 28 dBm, effectively one-ninth of the experimental diffusion thickness in quiescent solutions. Overall, these values are consistent with the general observation that diffusion layer thicknesses are expected to be on the order of 10–100 μm .⁴¹

Pu Uptake at Ultratrace Levels as Analyzed by Mass Spectrometry. In addition to the trace analyte levels (initially 11 nM) used in LSC experiments, we briefly investigated the use of acoustic streaming for ion exchange from solutions containing extremely low levels of Pu, using TIMS for sample analysis. The initial 20 μL of 7.5 M HNO_3 solution contained nominally 2.4 fg of ^{244}Pu at 0.5 pM concentration. The SAW power setting was set at 25 dBm as above. The Pu uptake results versus time are plotted in Figure 5, and the natural log plot for rate determinations is shown in the inset. In these experiments, just one data point was determined at each elapsed time. The initial data points (0.5–2 h) under static diffusion are somewhat higher than would be expected compared with the data points at zero and from 4 to 8 h. Nevertheless it is still clear at these concentrations that the Pu uptake is significantly faster with mixing than without. The maximum observed uptake, using a TIMS analysis approach that is selective for Pu(IV) (see the Experimental Section) was 98% for both the mixed and static samples.

(46) If the LSC data are not normalized to 99% of total uptake, the observed rate constants are about 40% lower, $2.7(2) \times 10^{-5}$ and $1.4(1) \times 10^{-4} \text{ s}^{-1}$ for unmixed and mixed uptake experiments, respectively, compared to $4.2(4) \times 10^{-5}$ and $2.5(2) \times 10^{-4} \text{ s}^{-1}$ after normalization. The conclusions are unchanged, as the ratio of mixed to unmixed is still about 5 (5.2 instead of 5.8), and the rate constants are similar to those obtained in the TIMS experiments, whether the LSC uptake data were normalized or not.

(47) *Knovel International Critical Tables of Numerical Data, Physics, Chemistry and Technology*, 1st electronic ed. [Online]; Knovel: Norwich, NY, 2003.

Table 2. Ion-Exchange Rates at Varying SAW Station Power Settings for ^{238}Pu Standard in 7.5 M HNO_3 Solution^a

SAW power (dBm)	% sorbed	T ($^{\circ}\text{C}$) ^b	viscosity (cP) ^c	D ($\text{m}^2 \text{s}^{-1}$) ^d	k_{observed} (s^{-1}) ^e	δ_{observed} (μm) ^f
0	17(4)	23.2	1.409	1.791×10^{-10}	1.06×10^{-4}	153
22	60(4)	23.8	1.412	1.792×10^{-10}	5.07×10^{-4}	32
25	67(3)	25.6	1.418	1.794×10^{-10}	6.23×10^{-4}	26
28	84(2)	39.7	1.470	1.812×10^{-10}	10.2×10^{-4}	16
31	84(4)	52.8	1.519 ^g	1.828×10^{-10}	10.1×10^{-4}	16

^a Contact time was constant at 30 min. ^b 30 min temperature average. ^c Interpolated from literature data (ref 47). ^d Estimated using eq 4 and 86 pm as the effective ionic radius for six-coordinate Pu(IV) (ref 51). ^e Calculated from $k_{\text{observed}} = -\ln(X)/t$, where X is the fraction of Pu in solution at time t (in seconds). ^f Estimated diffusion layer thickness at various power settings, calculated from $\delta = 3\sqrt{D}/r_0 V k_{\text{observed}}$. ^g Extrapolated from literature data (ref 47).

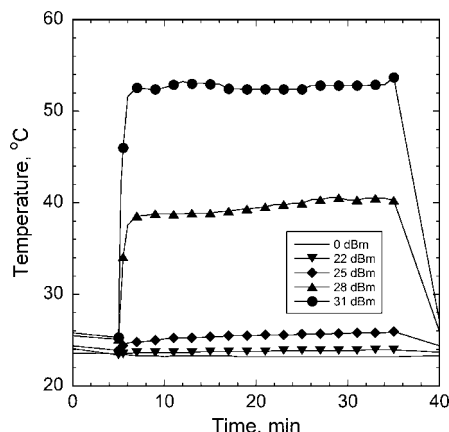


Figure 4. Temperature of 20 μL of deionized water in a microwell measured as a function of time at various SAW power settings. The SAW station was turned on at 5 min. Note that the temperature equilibrates ~ 2 min after SAW station activation.

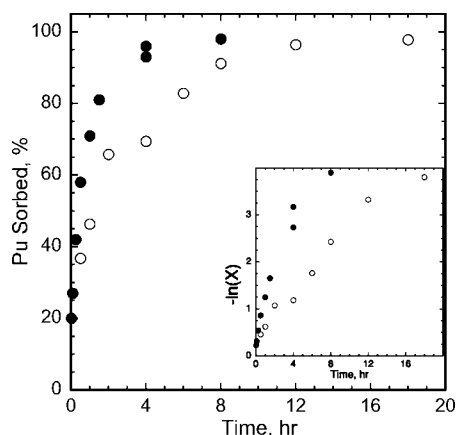


Figure 5. Percentage of $^{244}\text{Pu}(\text{IV})$ sorbed from solution as a function of time with (●) and without (○) acoustic streaming at 25 dBm in a sealed microwell, as measured by thermal ionization mass spectrometry (TIMS). Inset: Plot of the negative natural logarithm of the fraction, X , of ^{244}Pu in solution vs time with and without acoustic streaming.

The corresponding rate constants calculated by linear regression of the data in Figure 5, and shown in Table 1, were $0.59(5) \times 10^{-4} \text{ s}^{-1}$ and $1.8(1) \times 10^{-4} \text{ s}^{-1}$ for static diffusion and mixed samples, respectively. By this criterion, acoustic mixing provided a 3.1-fold increase in the observed rate constant. The smaller ratio of observed rate constants may be biased by the seemingly high initial data points under static conditions.

Discussion. Our results indicate that the ion-exchange kinetics can be significantly accelerated relative to static diffusion in

384-well plates with semiconical wells by using SAW devices to implement an acoustic streaming method of solution mixing. The acoustic streaming approach is a noncontact method that cannot contaminate the sample. Mixing results from the transmission of acoustic energy through the container walls, and it can operate on very small liquid volumes.

With the use of relatively moderate power settings (25 dBm), the rate of Pu uptake may be accelerated by a factor of 5, with only a slight increase in temperature ($< 2^{\circ}\text{C}$, stable over 30 min), based on our experiments using LSC for measurements. Significant acceleration (at least a factor of 3) was also observed in our initial experiments at subpicomolar concentrations using TIMS analysis. If samples can tolerate modest temperature increases and the equilibrium point is not lowered too much by higher temperatures, then higher power settings offer up to a ca. 9-fold rate constant increase over diffusion alone. We concluded that this accelerating effect at higher power settings is not due to the observed increase in temperature but rather the decrease in the diffusion layer thickness as a result of fluid velocities induced by mixing.

The acceleration we observe is consistent with that observed in other experimental systems using acoustic streaming methods to overcome diffusional limitations in static solutions. Schueller and Yang examined the adsorption of phenol from 100 mL solutions onto 0.5 g scale quantities of particulate carbon placed 7.6 cm from a 1 MHz ultrasonic transducer and reported mass transfer coefficients with acoustic streaming that were 7.8 times greater with mixing than without.²⁴ In these large-volume experiments, the acoustic streaming method provided similar rate enhancements to those obtained by mechanical stirring but without contacting a device directly with the sample. Liu et al. examined DNA hybridization on an electrochemical platform to observe the kinetics with and without mixing using a cavitation streaming method at 5 kHz and observed that hybridization was complete in 5.3-fold less time with mixing than without.²⁶

Our study shows that acoustic mixing can be used to accelerate small-volume (e.g., 20 μL in our case) heterogeneous batch uptake processes such as analyte capture on a single bead. We anticipate that, in the field of environmental analysis of actinides and other trace elements, this approach can be used to accelerate single-bead anion-exchange microchemical purification steps^{28–31} and single resin bead uptake for TIMS ion source preparation.^{29,30,32–38} Static diffusion requires 8 or more hours to achieve 95% uptake, so approaching complete equilibration would require more than 1 working day. With acoustic streaming, the equilibration could

be carried out in a half a day or less at 25 dBm or even faster at higher power settings.

Given a method to make small-volume single-bead uptake occur over reasonably short time frames, small batch single-bead uptake methods may be more feasible for additional analytical applications. For example, it follows that acoustic streaming could be used to accelerate analyte uptake on other solid-phase separation materials, such as extraction chromatographic materials,^{40,48–50} if separations in small batch scales are desired. More generally, the ability to thin the diffusion layer and accelerate reactions at

surfaces may be useful in electrochemistry, sensors, and the assembly of molecules on surfaces (e.g., self-assembled monolayers).

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