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Highly Porous Acrylonitrile-Based Submicron Particles for UO_2^{2+} Absorption in an Immunosensor Assay

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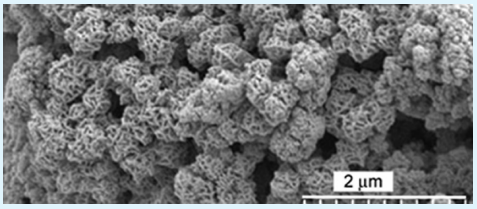
ABSTRACT: Our laboratory has previously reported an antibody-based assay for hexavalent uranium (UO_2^{2+}) that could be used on-site to rapidly assess uranium contamination in environmental water samples (Melton, S. J.; et al. *Environ. Sci. Technol.* **2009**, *43*, 6703−6709). To extend the utility of this assay to less-characterized sites of uranium contamination, we required a uranium-specific adsorbent that would rapidly remove the uranium from groundwater samples, while leaving the concentrations of other ions in the groundwater relatively unaltered. This study describes the development of hydrogel particles containing amidoxime groups that can rapidly and selectively facilitate the uptake of uranyl ions. A miniemulsion polymerization technique using SDS micelles was employed for the preparation of the hydrogel as linked submicrometer particles. In polymerization, acrylonitrile was used as the initial monomer, ethylene glycol dimethacrylate as the crosslinker and 2-hydroxymethacrylate, 1-vinyl-2-pyrrolidone, acrylic acid, or methacrylic acid were added as co-monomers after the initial seed polymerization of acrylonitrle. The particles were characterized by transmission electron spectroscopy, scanning electron microscopy (SEM) and cryo-SEM. The amidoximated particles were superior to a commercially available resin in their ability to rapidly remove dissolved UO_2^{2+} from spiked groundwater samples.



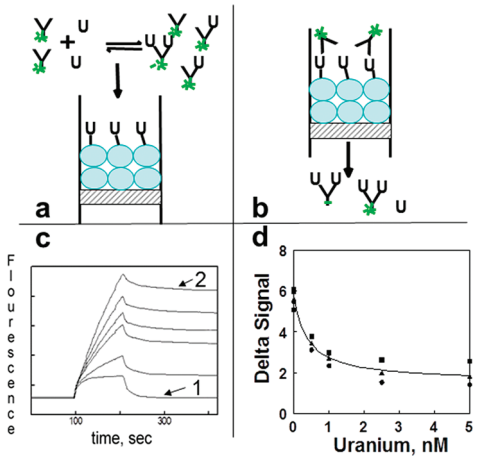
KEYWORDS: *hydrogels, nanoparticles, heavy metal removal, uranium absorption*

1. INTRODUCTION

The contamination of the environment with uranium can occur as a result of leaching from natural deposits, release from mine tailings, via emissions from the nuclear industry, during the combustion of coal and other fuels and from the use of phosphate fertilizers.[1] Military use of depleted uranium also releases material that can contaminate water supplies.[2] A variety of synthetic resins have been utilized for the removal of uranium from groundwater. In samples at very low pH (<3.8), Dowex 1-X8, Purolite A520E, and microporous poly(4-vinylpyridine) resins have been reported to efficiently absorb uranium.[3,4] At near-neutral pHs, Chelex 100, Dowex 21K and PANSIL have all shown utility in the removal of uranium from spiked artificial groundwater and contaminated environmental samples.[5−7] Diphonix resin, which has both ion exchange and chelating properties, has been shown to remove uranium at both near-neutral and acidic pHs.[8] The resins reported to be most effective at near neutral pHs have functional groups supported on styrene or silica particles.[9,10] Although these supports provide excellent mechanical strength, the nonporous nature of these particles limits the kinetics of uranium absorption.

In our laboratory, the need for a resin that could very rapidly remove uranium from environmental water samples arose during the development of antibody-based sensors for hexavalent uranium (U(VI) or UO_2^{2+}). The operation of these antibody-based sensors, which are based on the principal of kinetic exclusion,[11] is shown in Scheme 1. The antibody (Y* in Scheme 1) and U(VI)-chelate complexes derived from an

Scheme 1. Operation of the Uranium Immunosensor



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environmental sample were allowed to incubate until the binding reaction reached equilibrium (1 min or less, panel a), and the reaction mixture was then exposed briefly to chelated uranium immobilized on the surface of beads packed into a flow/observation cell installed in the sensor. Antibody molecules with no chelated U(VI) in their binding sites bound to the beads while antibodies already bound to the environmental uranium-chelate complexes were washed from the bead pack (panel b). Panel c shows representative traces of fluorescence versus time when mixtures containing the same concentration of antibody and varying concentrations of chelated uranium were analyzed. The immunosensor continually measured the fluorescent signal from the flow/observation cell during sample analysis; each run consisted of autonomous mixing of reagents (0−90 s), the injection of the antibody-chelated uranium mixture onto the flow/observation cell (90−200 s) and a buffer wash (200−410 s). If the environmental sample contained high uranium concentrations (trace 1), all of the antibody binding sites were already full and very little antibody was retained by the beads in the flow/observation cell. On the other hand, if the solution uranium concentration was low, most of the antibodies were available to bind to the beads and the fluorescent signal at the end of the run was high (trace 2). The difference in the fluorescence at the beginning (0−10 s) and the end (400−410 s) of each run was defined as the Delta Signal. A plot of Delta Signal versus the solution uranium concentration (panel d) was used as a calibration curve to quantify the uranium ion concentration in an environmental sample.

As with many assays that are based on biological recognition events (see ref 12 for a review) , the binding properties of the antibody incorporated into these sensors were influenced by components in the environmental sample matrix. The 12F6 monoclonal antibody used in our sensors binds tightly and specifically to chelated uranium;[13] however, its binding was slightly depressed by the very high concentrations of calcium present in groundwater. This interference did not invalidate the assay for screening purposes, but we discovered that assay precision and accuracy could be improved if we adjusted our calibrators to have an ionic composition similar to that in the samples being analyzed. In our previous studies, the general ionic composition of the groundwater at the test site (including the average calcium concentration) was known and when we amended our calibrators to include ions from the groundwater, the accuracy and precision of the antibody-based assay was as good as that available from the "gold standard" for uranium analysis, kinetic phosphorescence analysis.[14,15]

To extend the operation of the antibody-based sensors to less characterized sites, we required a resin that could rapidly (<5 min) and specifically absorb the virtually all of the uranium from an environmental water sample, while leaving other ions (especially calcium ions) in the treated sample. Such an adsorbent could be used to prepare a "uranium-free" sample matrix for use during instrument calibration, similar to the artificial groundwater formulations used in our previous experiments.[14] These resins could also be used to concentrate uranium in very dilute samples (after a specific elution step). Although commercial actinide-specific resin-based adsorbents were available,[16,17] our preliminary tests showed that they required almost 60 min to adsorb the uranium from groundwater samples, even with a high resin/sample ratio. Our current work was therefore directed toward the development of alternate hydrogel-based materials for the rapid and selective uptake of uranium.

Hydrogels have been investigated intensively over last several decades for a variety of applications in biological and environmental materials, both because of their ability to take up significant amounts of water and the fact that their chemical structure can be finely tuned for specific applications.[18−23] The amidoxime group ($RC(=NOH)NH_2$) has been shown to be effective in chelating uranium[10,24] and our objective was to prepare submicrometer particles of hydrogel with this functional group to facilitate rapid and selective uptake of uranium ions. The synthesis and characterization of these materials is the focus of this report.

2. EXPERIMENTAL METHODS

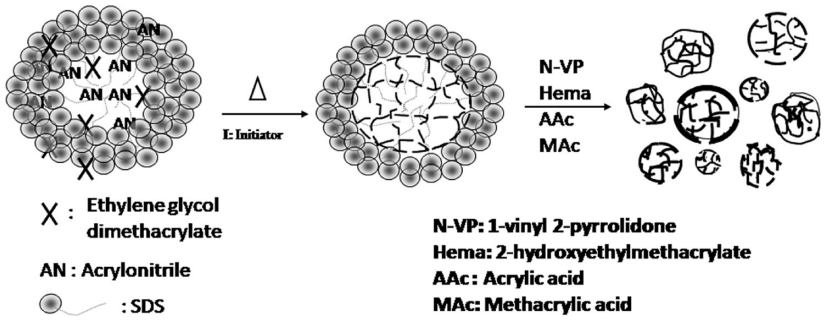
2.1. Materials. The monomers, acrylonitrile (AN, 99+%), 1-vinyl-2-pyrrolidone (1-VP, 99+%), 2-hydroxy ethylmethacrylate (HEMA, 97%), acrylic acid (AAc, 99%), methacrylic acid (MAc, 99%), the cross-linker, ethylene glycol dimethacrylate (EGDMA, 98%), the initiators, ammonium persulfate (APS, 98%) and 2,2-dimethoxy-2-phenyl-acetophenone (DPA), the salts used to prepare an artificial groundwater composite sample ($CaCl_2{\cdot}2H_20$; $CaSO_4{\cdot}2H_2O$; $Ca(NO_3)_2{\cdot}4H_2O$; $MgCl_2{\cdot}6H_2O$; $NaNO_3$; KCl, and $MnCl_2{\cdot}4H_2O$) and the hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, 98%) used for the amidoximation reaction were obtained from Sigma-Aldrich Chem. Co. (Milwaukee, Wisconsin) and used as received. The surfactant, sodium dodecyl sulfate (SDS) (≤98%) was purchased from Bio-Rad Laboratories (Hercules, CA). Purified water (18.3 megaohm-cm) from a Nanopure Diamond purifier (Barnstead) was used for preparation of all aqueous solutions. Monoclonal antibodies with specificity for chelated uranyl ions, the chelator, 2,9-dicarboxyl-1,10-phenanthroline (DCP) and a DCP-bovine serum albumin conjugate were available from previous studies.[13,14,25] A Cy5-labeled Fab fragment of goat anti-mouse IgG was a product of Jackson ImmunoResearch Laboratories (West Grove, PA). TRU and UTEVA resins were purchased from Eichrom (Darien, IL). IC Millex filter units (13 mm diameter, 0.2 μm pore size) were a product of Millipore, Inc. (Billerica, MA). Uranyl acetate used to spike groundwater samples was a product of Mallinckrodt, Inc. (Hazelwood, MO).

2.2. Synthesis of Submicrometer Particles. A miniemulsion system was used for the synthesis of submicrometer hydrogel particles, as shown in Scheme 2. The relatively hydrophobic acrylonitrile (AN) monomer was loaded into micelles of sodium dodecyl sulfate (SDS) in aqueous solution and simultaneously crossed-linked and polymerized. In a typical experiment, 0.3 mL of AN was dispersed in 15 mL of 0.1 M SDS aqueous solution. To this solution was added the cross-linking agent, ethylene glycol dimethacrylate (EGDMA, 2.5−10% based on AN monomer mole ratio). The mixture was vortexed until a clear solution was obtained. The simultaneous polymerization and cross-linking reaction was initiated by the addition of ammonium persulfate (APS, 1.25% based on moles of AN, dissolved in 1 mL of water). The reaction proceeded under constant mixing (750 rpm) at 75 °C for 10 h. The particles formed during the reaction were then washed by adding an excess amount of water and centrifuging at 4000 rpm for 10 min. The exhaustive washing with water (at least 10 times) removed virtually all the surfactant. The particles were lyophilized after water washing.

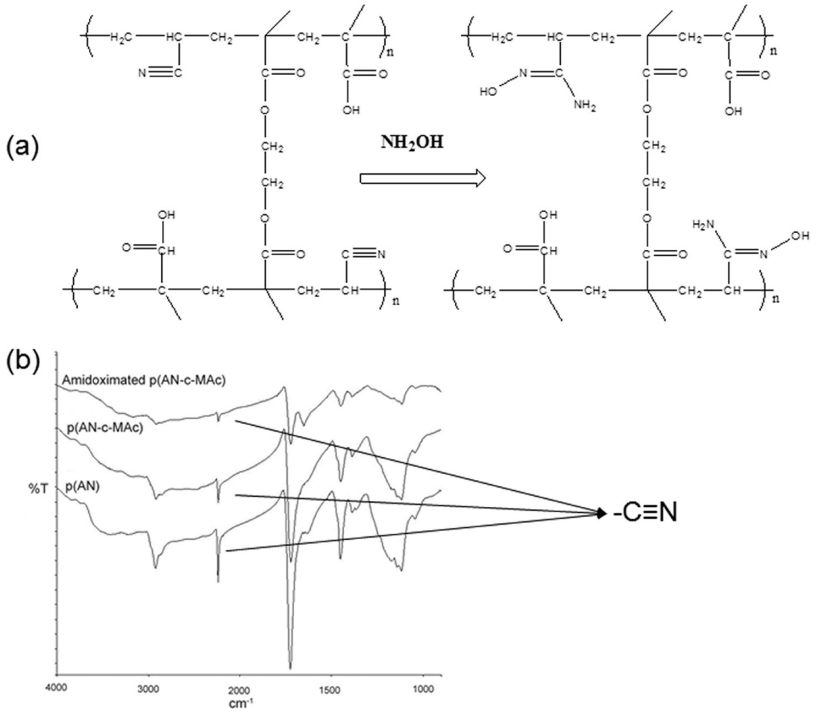
For copolymer-based particle synthesis, the AN monomer was loaded into SDS micelles and crosslinker EGDMA and initiator APS were added as described above. After reaction for 2 h at 75 °C, other monomers, including 1-vinyl-2-pyrrolidone (1-VP), 2-hydroxy ethyl-methacrylate (HEMA), methacrylic acid (MAc), or acrylic acid (AAc) were added to the reaction mixture in two different molar ratios with respect to AN (0.33 and 0.5). After vortexing thoroughly, these mixtures were allowed to react for an additional 8 h at 75 °C under constant mixing (750 rpm). The same washing procedure was followed as described above. The particles were lyophilized after water washing; the lyophilized particles were stored in a vacuum oven at ambient temperature for later use.

2.3. Amidoximation. The nitrile groups on the acrylonitrile moieties of the p(AN-c-HEMA) and p(AN-c-MAc) copolymeric

Scheme 2. Miniemulsion System for Synthesis of Submicrometer Hydrogel Particles



Scheme 3. (a) Amidoximation Reaction Scheme of p(AN-c-MAc) and Structure of the Final Hydrogel; (b) FT-IR Spectra of p(AN), p(AN-c-MAc), and Am-p(AN-c-MAc)



particles were converted to amidoxime groups in an aqueous environment, as shown in Scheme 3. The lyophilized particles were weighed (1.365 g) and the moles of AN contained in these particles was calculated based on the assumption that 100% the AN in the feed had been polymerized. Hydroxylamine hydrochloride (5-fold molar excess to p(AN)) was added to 200 mL of water and neutralized with NaOH. The particles were subsequently added to this solution and the reaction mixture was stirred (750 rpm) for 24 h at 25 °C. After the amidoximation reaction, the low molecular weight contaminants were removed from the polymer particles by 5 washes in an excess of water; the particles were centrifuged at 4000 rpm for 10 min before removing the supernatant. The amidoximation reaction was verified via Fourier Transform Infrared Radiation (FT-IR) spectroscopy with a PerkinElmer FT-IR 1600 System Spectrum GX. The loss of the nitrile groups was used as evidence of the conversion of nitrile to amidoxime.

2.4. Microstructure Analysis. Transmission electron microscopy (TEM) was performed using a JEOL 2010 scanning transmission electron microscope. Cleaned virgin and amidoximated particles were placed in Nanopure water and ultrasonicated about 5 min. A small drop of the sample solution was dropped onto a 200 mesh carbon-coated copper grid with formvar film, and dried in air. The samples were observed in vacuum at an accelerating voltage of 200 keV at room temperature.

Scanning electron microscopy (SEM) was performed using a Hitachi S-4800 field-emission electron microscope. A suspension of the AN-based particles in nanopure water was added to carbon tape attached to SEM stubs. The particles were dried at ambient temperature and sputter coated with ∼5 nm of platinum/gold. SEM images were acquired at an operating voltage of 10 keV.

Cryo-SEM was performed on particles that had been allowed to swell overnight in Nanopure water. The water-swollen particles were mounted on the sample stage and rapidly plunged into liquid nitrogen slush at approximately −190 °C (Gatan, Alto 2500). The sample was withdrawn into a vacuum transfer device under the protection of high vacuum and transferred into the cryo-preparation chamber, where the temperature was maintained at −130 °C, with the anticontaminator at ∼ −188 °C. The sample was sublimated for 5−10 min at −95 °C to etch away surface water, then the temperature of the stage was adjusted back to −130 °C and the sample was sputter coated with platinum at 10 mA for 100 s. The sample was subsequently transferred into the main chamber of a field emission SEM (Hitachi S-4800) via an interlocked airlock and mounted onto a cold stage module (−130 °C) fitted to the SEM stage. Images were acquired at a voltage of 3 keV and at a working distance of 8 to 13 mm.

2.5. Treatment of Uranium-Spiked Groundwater Samples with Submicrometer Particles and Measurement of UO_2^{2+} Using an Immunosensor-Based Assay. Artificial groundwater with

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inorganic constituents similar to that at an uncontaminated site at Oak Ridge National Laboratory was prepared based on a formulation provided by Dr. Scott Brooks, Subsurface Science Group, Environmental Sciences Division, ORNL, Oak Ridge, TN. This artificial groundwater composite contained the following cations and anions: Ca^{2+}, 4.07 mM; Mg^{2+}, 0.107 mM; Na^{+}, 0.076 mM; K^{+}, 0.016 mM; Mn^{2+}, 0.002 mM; Cl^{-}, 3.938 mM; SO_4^{-2}, 1.561 mM; NO_3^{-}, 1.383 mM. The pH of this formulation was 5.9. This artificial groundwater composite was subsequently spiked with 30 ppb (126 nM) or 1 ppm (4.2 μM) of UO_2^{2+} (as uranyl acetate).

To determine the time course of uranium absorption, lyophilized particles synthesized as described above or the commercially available TRU resin were weighed into 2 mL vials and the spiked artificial groundwater was added to achieve a final concentration of 16.7 mg of absorbing material per mL of the spiked groundwater. The samples were mixed to suspend the resins and the absorption was stopped by filtration of each sample through an IC Millex filter unit after varying times in contact with the particles. A spiked groundwater composite sample that had not been in contact with the resins was used as the control for these experiments.

The volume of the filtered solution was measured and mixed with an equal volume of a 2× concentrated Hepes-buffered saline stock solution containing 400 nM 2,9-dicarboxyl-1,10-phenanthroline (DCP) such that the final mixture contained 137 mM NaCl, 3 mM KCl, 10 mM Hepes buffer, pH 7.4, 50% of the UO_2^{2+}-spiked groundwater sample and 200 nM DCP. The samples were further diluted with Hepes-buffered saline (HBS, 137 mM NaCl, 3 mM KCl, 10 mM Hepes, pH 7.4) containing 200 nM DCP to reach the working range of the Inline Immunosensor (0.25-6.0 nM UO_2^{2+}). The UO_2^{2+} content in the control and each experimental sample was analyzed by a previously published procedure[14] in an assay mixture that contained 0.25 nM monoclonal antibody 12F6,[13] 200 nM DCP, and 5 nM Cy5-labeled Fab fragment of goat anti-mouse IgG in HBS. Samples were mixed and immunoassays were run autonomously in an automated Inline Immunosensor described previously by our laboratory[13] and available from Sapidyne Instruments (Boise ID). UO_2^{2+} was also measured using kinetic phosphorescence analysis and Uraplex reagent.[26]

The capacity of the amidoximated p(AN-c-MAc) particles for UO_2^{2+} absorption was tested by adding varying concentrations of Am-p(AN-c-MAc) particles (16.7, 1.67, 0.167, and 0.0167 mg/mL) to artificial groundwater spiked with 1 ppm of UO_2^{2+}. After 5 min of mixing in the presence of the particles, the samples were filtered and the UO_2^{2+} remaining in the solution was measured as described above.

2.6. Determination of Cation Selectivity. Cation selectivity was determined by performing inductively coupled plasma emission spectroscopy (ICP) on the UO_2^{2+}-spiked groundwater samples (described above) that had been treated for 5 minutes with 16.7 or 0.167 mg/mL of the Am-p(AN-c-MAc) particles. ICP was performed at the Tulane Coordinated Instrument Facility using a Perkin Elmer Optima 3000 inductively-coupled plasma atomic emission spectrometer.

2.7. Preparation of a "Matrix Blank" for Immunoassay. An acidified environmental sample containing 650 nM of UO_2^{2+} (155 ppb) was available from a previous study.[14] The sample was neutralized to ~pH 7.0 with a small volume of 8 M KOH and Am-p(AN-c-MAc) particles were added to a concentration of 16.7 mg/mL. The sample was mixed for 5 minutes and the beads were removed by filtration through an IC Millex-LG filter unit. Immunoassay standard curves were prepared using either 1% artificial groundwater or 1% Am-p(AN-cMAc)-treated environmental groundwater and the following concentrations of UO_2^{2+}: 0, 0.6 1.2, 2.25, and 6 nM. Each assay standard also contained 0.25 nM monoclonal antibody 12F6,[13] 200 nM DCP, and 5 nM Cy5-labeled Fab fragment of goat anti-mouse IgG in HBS. The delta signals determined at each uranium concentration were fit to a curve using SlideWrite software (Advanced Graphics Software, Carlsbad, CA) and the following equation: $y = a_0 - (a_1x)/(a_2 + x)$, where a_0 is the delta when no UO_2^{2+} is present in the sample (y intercept), a_2 is the UO_2^{2+} concentration that provides a 50% decrease in the maximum signal, and a_1 is the total change in the value of delta as x goes from zero to infinity. The curve fit software also provides 95% confidence intervals for the constants a_0, a_1, and a_2, which can be used in the comparisons of different standard curves.

3. RESULTS AND DISCUSSION

The use of bulk hydrogels for the absorption of radionuclides from groundwater samples had been established in earlier work by Sahiner and coworkers.[24,27] Although these bulk gels were effective in removal of soluble UO_2^{2+} from environmental samples, the time required for the process (>60 minutes) was too slow for use in a near real-time, sensor-based assay. Because hydrogel particles are smaller in size and have a higher surface area per unit volume than bulk hydrogels, they have superior absorption kinetics. A miniemulsion method was therefore used for the preparation of sub-micrometer size hydrogel particles, as shown in Scheme 2. In the emulsion system used herein, AN resided primarily in the hydrophobic center of SDS micelles because its solubility in water was relatively low (80 g/L).[28,29] Concurrent addition of comonomers such as VP and AAc for copolymer particle synthesis resulted in gel formation at high feed ratios ($V_{AN} \geq 0.4$ mL and mole ratios of AN to comonomers 1:1). The particle synthesis was therefore initiated by the synthesis of acrylonitrile (AN)-based hydrogel seed particles. AN was chosen as the seed material because the cyano (nitrile) groups in AN provided facile sites for further functionalization to the amidoxime group. After 2 h of polymerization, these seed particles were modified by adding hydrophilic monomers such as 1-vinyl-2-pyrrolidone (1-VP), 2-hydroxy ethylmethacrylate (HEMA), methacrylic acid (MAc), or acrylic acid (AAc), in order to prepare particles that were able to rapidly absorb water and swell. It was also possible to prepare particles without the use of a seed polymerization technique by lowering the feed ratios of the hydrophilic monomers and keeping the feed amounts of monomers very low. Finally, the nitrile groups on the acrylonitrile moieties of the copolymeric particles were converted to amidoxime groups. Scheme 3 illustrates the transformation of acrylonitrile groups to amidoximine groups in the final hydrogel and shows the FT-IR spectra that demonstrate loss of the nitrile groups, which was used as evidence of the conversion of nitrile to amidoxime.

From our previous investigations, we found that AN's polymerization and crosslinking was approximately 50% at 75 °C for 2 h reaction time with 1% cross-linker ratio based on AN amount.[30] On the basis of this earlier work, and on the somewhat higher amount of cross-linker used in the present study (2.5 to 10% based on AN amount), we allowed the AN to polymerize for 2 h before the addition of any co-monomers (VP, HEMA, AAc, or MAc). This allowed some seed p(AN) particles to form before the addition of comonomers. The reaction was then continued for 8 additional h after the addition of comonomers. Unless otherwise stated, the ratio of comonomer to AN was 1−3 based on AN amount for all the copolymeric particle syntheses. p(AN) particles without the comonomer shell were also prepared for comparison; these p(AN) particles were prepared with different % cross-linker (EGDMA) ratios at 75°C and a 10 h reaction time. Table 1 summarizes the different conditions used during synthesis and provides a description of the abbreviations used for the particles described in this report.

To understand the topographic features of the particles, we performed TEM, SEM, and cryo-SEM studies. TEM of p(AN) particles without a copolymer shell are shown in images a and b in Figure 1. These panels show particles made using 5 and 10% cross-linker ratios, respectively. Both conditions yielded particles

Table 1. Synthesis of Particles Used in This Study[a]

particle type	% EGDMA[b]	AN/ coating monomer[c]	description
p(AN)	5 and 10	N/A	acrylonitrile particles
p(AN-c-MAc)	5 and 10	2 and 3	acrylonitrile particles with a coating of polymerized methyl acrylic acid
p(AN-c-HEMA)	2.5 and 5	2 and 3	acrylonitrile particles with a coating of polymerized 2-hydroxy ethylmethacrylate
p(AN-c-VP)	5 and 10	2 and 3	acrylonitrile particles with a coating of polymerized 1-vinyl-2-pyrrolidone
p(AN-c-AAc)	5 and 10	2 and 3	acrylonitrile particles with a coating of polymerized acrylic acid
Am-p(AN-c-MAc)	5 and **10**	2 and **3**	amidoximated acrylonitrile particles with a coating of polymerized methyl acrylic acid
Am-p(AN-c-HEMA)	2.5 and **5**	**2** and 3	amidoximated acrylonitrile particles with a coating of polymerized 2-hydroxy ethylmethacrylate

[a]Synthetic conditions used for the particles described in Figure 4 and Table 2 are shown in bold type. [b]EGDMA, ethylene glycol dimethacrylate. Values are reported as mol % cross-linker used. [c]Values are reported as the mole acrylonitrile/mol of hydrophilic monomer.

with an interesting wrinkled architecture. The particulate form of the polymer is a consequence of synthesis in a microemulsion system, and collisions between the microemulsion droplets lead to highly interconnected particles. The voids between the particles are indicative of enhanced surface area compared to a bulk polymer. The addition of a copolymer shell composed of MAc (to form p(AN-cMAc particles), shown in panel c, makes the morphology more diffuse. This diffuse structure is also observed upon amidoximation (Am-p(AN-c-MAc) particles, panel d). We observed that the hydrophilicity enhancement upon amidoximation also facilitates spreading of the particles on a TEM grid, although there are insignificant morphological distinctions between amidoximated and non-amidoximated particles seen on the TEM. For the copolymeric particles prepared from other monomers (VP, HEMA, AAc) similar diffuse structures were also observed after amidoximation (data not shown).

The SEM images of 10% cross-linked p(AN) and 10% cross-linked p(AN-c-MAc), shown in images a and b in Figure 2, respectively, indicate the highly porous structures of the interconnected polymer particles. The particles of the nonmodified p(AN), shown in panel a, appear to have the skeletal structure that is also observed in the TEMs of Figures 1a and 3b. In order to image the hydrated state of the particles, we used cryo-SEM where the hydrated sample is vitrified prior to analysis. Figure 3 illustrates the high-resolution cryo-SEM images of cross-linked p(AN) (a) and p(AN-c-MAc) particles (b) prior to amidoximation, indicating that the polymer morphology is that of linked particulates, rationalized by the mini-emulsion method used in the synthesis. These images also suggest that the hydrated particles are swollen compared to samples that had undergone the critical point drying required by conventional SEM.

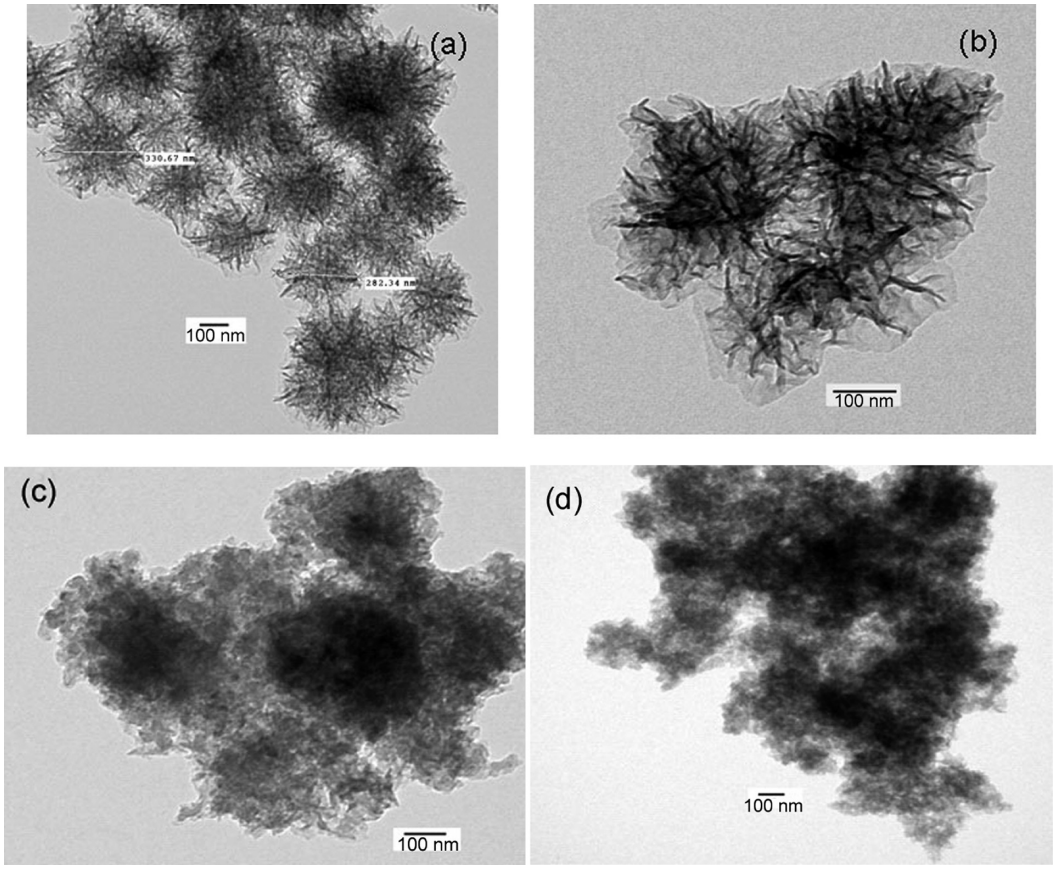

Figure 1. TEM images representing (a, b) 5% cross-linked and 10% cross-linked p(AN) particles, respectively, before addition of the hydrophilic copolymer material;(c) 10% cross-linked acrylonitrile particles, coated with a shell of polymerized methacrylic acid, p(AN-c-MAc); and (d) final polymer of amidoximated 10% cross-linked p(AN-c-MAc) particles.

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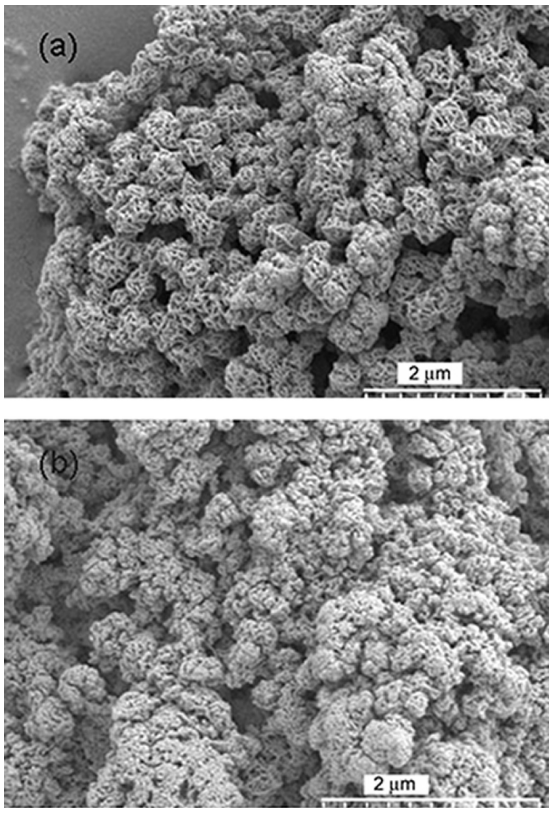

Figure 2. SEM images of (a) 10% cross-linked p(AN) and (b) 10% cross-linked p(AN-c-MAc) particles. These images demonstrate the porosity and high surface area of these particles.

The amidoximated particles were designed to be included as part of an immunoassay kit for soluble UO_2^{2+},[14,15] where the particles will be used to generate a groundwater sample from which all the UO_2^{2+} has been removed. Thus, the hydrogel particles were compared to a commercially available resin for their ability to rapidly and selectively remove uranium from a UO_2^{2+}-spiked groundwater sample. Preliminary experiments were performed to compare two commercially available U-binding resins, UTEVA and TRU. The TRU resin showed faster kinetics in our experimental protocol (comparison data not shown) and this resin was used for all comparisons with the hydrogel-based particles. In the analysis, filtration through a 0.2 μm filter was sufficient to remove both the commercial resin and the AN-based particles from the spiked groundwater samples. The kinetics of UO_2^{2+} removal was then directly compared for the hydrogel based particles and the TRU® resin, as shown in Figure 4. The data in panel a show the removal of UO_2^{2+} spiked at an initial concentration of 30 ppb (126 nM), whereas the data in panel b compares the ability of the amidoximated particles and the TRU resin to remove uranium from a sample spiked at 1 ppm (4.2 μM). As expected, when the hydrogel particles were added at the same weight/volume as the TRU resin, they absorbed UO_2^{2+} much more rapidly; virtually all of UO_2^{2+} was removed from the 30 ppb spiked sample by the time we had collected the first time point (1 minute after addition). When the TRU resin and the MAc particles were compared at the 1 ppm concentration, the hydrogel particles again removed the uranium to below the level of detection in the assay after 5 min of incubation. A 5 min time point was utilized for these experiments because it represented a convenient time period for a field treatment protocol. The TRU resin showed approximately the same kinetics of uranium removal in both spiked samples, removing ~75% of total uranium at the 5 min time point and 85% after 30 min of incubation with the sample. Comparable results on the removal of uranium from the treated samples by the TRU resin and hydrogel particles was obtained using kinetic phosphorescence analysis (data not shown).

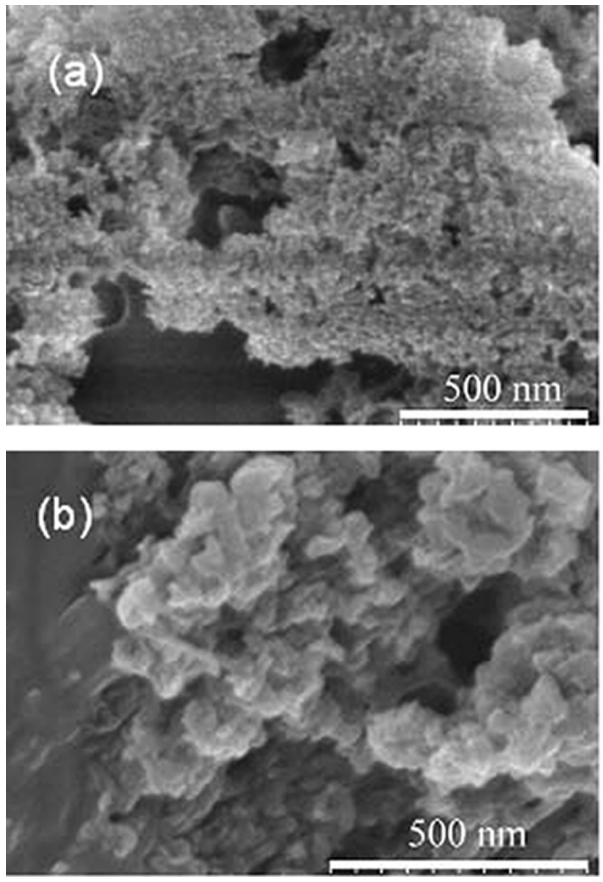

Figure 3. Cryo-SEM images of (a) 10% cross-linked p(AN) and (b) 10% cross-linked p(AN-c-MAc) particles.

Experiments were subsequently performed to estimate the UO_2^{2+}-binding capacity of the Am-p(AN-c-MAc) particle preparation. When groundwater spiked with 1 ppm of UO_2^{2+} was treated for 5 min with the lyophylized particles at 16.7, 1.67, and 0.167 mg of dry weight/mL of sample, the uranium in each treated and filtered sample was below the limit of detection of our immunoassay (0.25 nM or 0.06 ppb). The capacity of the Am-p(AN-c-MAc) particles for uranyl ions was exceeded when the particle concentration was lowered an additional 10-fold, to 0.0167 mg/mL. At this low particle concentration, only 86% of the uranium in the groundwater sample was removed after 5 min of treatment. If we assume that the particles were completely saturated with uranium under these conditions, then the uranium binding capacity (mg/g particle) can be estimated as follows: 0.86 μg uranium/0.0167 mg particles = 51.5 μg uranium/mg particles or 51.5 mg U/g particles. On the basis of these data, we estimated the binding capacity of Am-p(AN-c-MAc) particles to be 51.5 mg of uranium per gram dry weight of the particles.

The cation selectivity of the Am-p(AN-c-MAc) particles was investigated by determining the concentrations of Na, Mg, Mn, Ca, and K before and after treatment with the particles, as

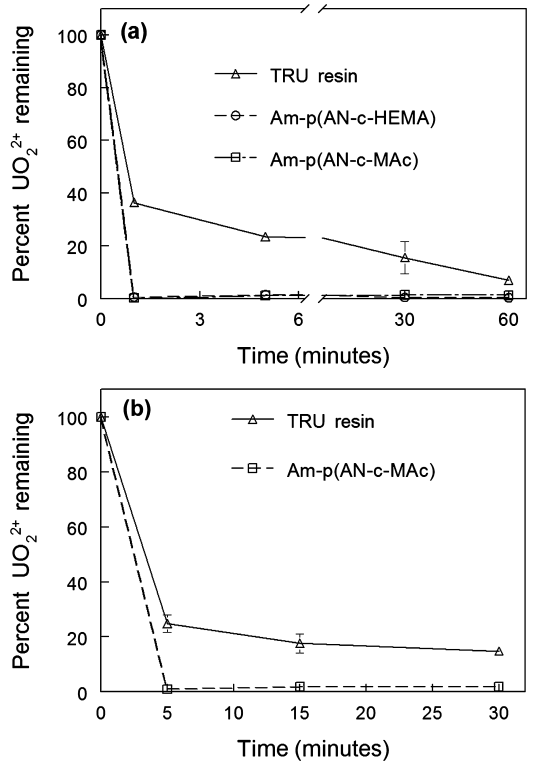

Figure 4. Time-dependence of uranium removal from spiked groundwater samples: (a) uranium removal from a solution containing 30 ppb of uranium, (b) uranium removal from a solution containing 1 ppm of uranium. Data are reported as the mean ± SD (n = 3). In some cases, the error in the analysis was less than the diameter of the plotted points.

shown in Table 2. Treatment of the spiked groundwater samples with 0.167 mg/mL of the particles (the lowest

Table 2. Cation[a] Selectivity of Am-p(AN-c-MAc) Particles

sample	Na	Mg	Ca	Mn	K
control	2461 ± 126	2480 ± 11	172128 ± 113	106 ± 3	567 ± 118
treated with 0.167 mg/mL particles	1055 ± 131	2433 ± 61	181538 ± 6533	108 ± 4	640 ± 27
treated with 16.7 mg/mL particles	nd[b]	1871 ± 94	98811 ± 155	4 ± 0.2	693 ± 30

[a]Cation concentrations are reported as μg/L (ppb). [b]Not detected (below the blank value for the ICP).

concentration that completely removed the UO_2^{2+} from the sample) reduced the Na concentration by ~57% but had a negligible effect on the other major cations in the sample. If we added particles in 100-fold excess of what was required to remove the UO_2^{2+} (16.7 mg/mL) then, in addition to the UO_2^{2+}, the particles also completely removed the Na^+ from the sample and significantly reduced the Mg^{2+}, Ca^{2+}, and Mn^{2+}. The reduction in Na^+ observed in these studies will be negligible in the performance of the antibody-based assay, since the groundwater samples are diluted from 1:25 to 1:100 into a physiological buffer containing 137 mM Na (3151 ppb) before analysis in the immunosensor.[12,13]

Finally, the U-binding particles were used to generate a uranium-free matrix sample to use for uranium analysis. An acid-stabilized environmental groundwater sample, available from a previous study,[14] was neutralized and treated with Am-p(AN-cMAc) beads to generate an uranium-free sample matrix. When this sample was used instead of artificial groundwater to develop a uranium standard curve, the two standard curves generated in the analysis both fell between the 95% confidence limits for the experimental data, as shown in Figure 5.

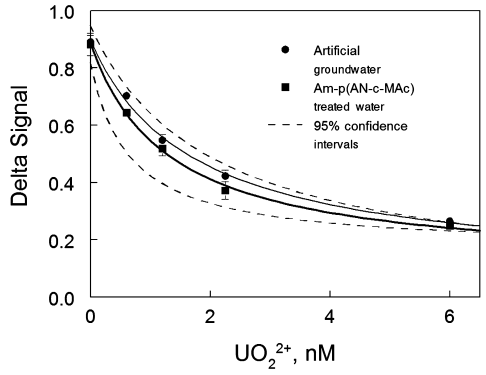

Figure 5. Uranium standard curves prepared with an artificial groundwater sample and with an environmental groundwater sample depleted of uranium with Am-p(AN-c-MAc) particles. Individual data points are plotted as the mean ± SD (n = 3). In some cases, the error in the analysis was less than the diameter of the plotted points. The dashed lines show the 95% confidence limits for the standard curves, as determined from the 95% confidence intervals for the constants, aO, al, and a2.

4. CONCLUSIONS

AN-based particles with diameters from nanometers to micrometers can be prepared by a simple oil-in-water emulsion method by employing a seed polymerization technique. The copolymeric particles of AN with VP, HEMA, AAc, and MAc can be readily prepared by this method and the procedure is an improvement over current synthetic methods. The AN-based particles show a porous structure with a high surface area. Amidoximated derivatives of the HEMA and MAc copolymeric particles are superior to a commercial resin in the rate of uranyl ion absorption. Treatment of a spiked groundwater composite sample for 5 minutes with as little as 167 mg dry weight/L of the p(AN-c-MAc) particles was sufficient to reduce the UO_2^{2+} concentration from 1 ppm to ≥0.06 ppb, with negligible effects on the concentrations of other divalent cations normally found in groundwater. Amidoximated copolymeric particles of AN with hydrophilic polymer shells may be useful for remediation of environmentally hazardous materials. Their very rapid uptake kinetics could make them extremely useful for water treatment processes that require high throughput. The translation of these materials to our immunosensor applications and the test of these particles with a wider range of uranium-contaminated environmental samples are topics of continued research in our laboratories.

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