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A Biocompatible Method of Decorporation: Bisphosphonate-Modified Magnetite Nanoparticles to Remove Uranyl Ions from Blood

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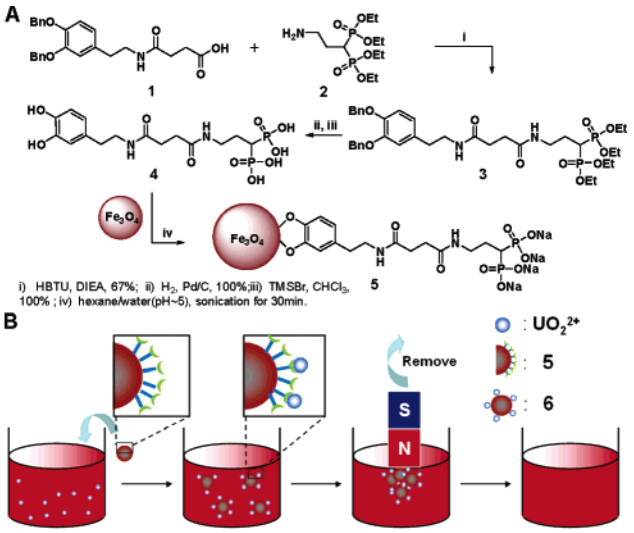
This communication describes the use of bisphosphonate-modified magnetite nanoparticles to remove radioactive metal toxins (e.g., UO_2^{2+}) with high efficiency from water or blood. Although metal ions (except cadmium, mercury, and lead) are essential for living cells, their accumulation beyond optimal concentrations in tissues or organs usually results in various diseases and disorders because they chelate to essential functional groups, displace other metal ions, or destroy the active conformation of biological molecules.[1] In addition to their metal toxicity, radioactive heavy metals, such as isotopes of uranium, cesium, and americium, could inflict radiological risks to human. Thus, it is important to develop a safe and effective procedure to remove radionuclides from the body (decorporation) after radiological contamination that would arise from either accidents or possible malicious attacks. The existing methods (e.g., ion exchange, chemical precipitation, membrane filtration, and liquid extraction) for the removal or recovery of heavy metal ions, including transuranics from aqueous phases, however, remain unsuitable for the purpose of decorporation. Despite its own limitation for in vivo use, the magnetically assisted chemical separation (MACS) process,[2] which utilizes magnetic microparticles coated with a selective extractant for the efficient recovery of radionuclides, provides a useful guide to develop a method of decorporation based on biocompatible magnetic nanoparticles.

The recent successful synthesis of monodispersed magnetic nanoparticles,[3,4] particularly iron oxide nanoparticles,[3,5] provides a basis on which to explore further applications of magnetic nanoparticles in biomedicine due to the inherent biocompatibility of iron oxide.[6] Several groups have demonstrated exciting and promising biological applications of magnetic nanoparticles[7,8] and confirmed superior performances of magnetic nanoparticles to magnetic microparticles in applications, such as separation of proteins and detection of pathogens.[8,9] These works, together with our result that bisphosphonate-containing hydrogel effectively lowers the toxicity of UO_2^{2+}-contaminated wounds on mice,[10] led us to develop a conjugate of dopamine and bisphosphonate (**4**, DA−BP, which binds tightly to iron oxide and coordinates to a uranyl ion (UO_2^{2+}) with high affinity) to modify magnetite Fe_3O_4 nanoparticles for decorporating UO_2^{2+}. Using the simple procedure illustrated in Scheme 1, we found that the designed magnetic nanoparticles, **5**, can remove 99 and 69% of UO_2^{2+} from water and blood, respectively. In addition to it being the first example of the removal of radionuclides from a biological fluid by nanoparticles, this result suggests that these functionalized, biocompatible magnetic nanoparticles can act as useful and effective agents of decorporation for selective and rapid removal of radioactive metal toxins in vivo.

Scheme 1. (A) Synthesis of the Surface-Modified Magnetite Nanoparticles and (B) the Removal of UO_2^{2+} from Blood



Scheme 1A shows the synthetic route for making the conjugate of **4** and Fe_3O_4 nanoparticles. Compound **1** reacted with **2** to give **3** with 67% yield after purification. Then **3** was further hydrogenated to remove the benzyl group and treated with bromotrimethylsilane (TMSBr) to eliminate diethyl ester to yield **4**. Then Fe_3O_4 nanoparticles (in hexane phase) reacted with **4** (in aqueous solution, pH ∼ 5) under vigorous stirring overnight or under sonication for 30 min to form Fe−O bonds that linked **4** to the Fe_3O_4. After the reaction, the resulting product, **5**, became water soluble and was easily separated from the organic phase for further characterization and testing.

As shown in the TEM images (Figure 1), **5** remains as crystalline nanocrystals (similar to the as-prepared Fe_3O_4 nanoparticles) and exhibits slight aggregation as the result of surface modification by the attachment of **4**. Time-of-flight second ion mass spectra (ToF-SIMS) confirm the DA−BP moieties on the surface of Fe_3O_4,[11] which displays not only the fragments of iron-coordinated **4** (FeO_2·**4**$^+$, m/e = 540) but also the fragment of the iron-coordinated dihydroxylbenzyl moiety ($FeO_2C_7H_6^+$, m/e = 178), further proving that **4** forms covalent bonds with Fe atoms on the surface of the Fe_3O_4 nanoparticles. The weight analysis[11] estimates an average of 54 molecules of **4** on one Fe_3O_4 nanoparticle.

Scheme 1B illustrates the procedure of using **5** to remove uranyl ions in water or blood, which consists of three major steps: mixing, binding, and removing/decorporating. Before using **5** to decorporate UO_2^{2+} from blood, we first tested the binding ability of **5** with uranyl ion in water ($[UO_2^{2+}] = 10^{-4}$ M). After being mixed with uranyl ion contaminated samples (i.e., UO_2^{2+} in water), **5** selectively coordinated with UO_2^{2+} to form **6** in water. A small bar magnet

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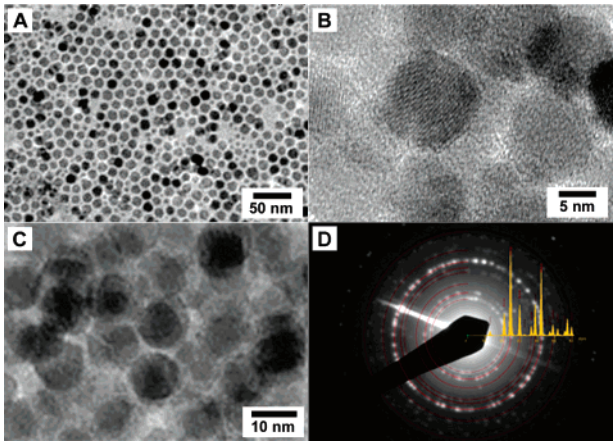

Figure 1. Transmission electron micrograph (TEM) images of (A) as-prepared Fe_3O_4 nanoparticles, (B) **5**, and (C) **6**; (D) electron diffraction patterns (EDP) of **6**.

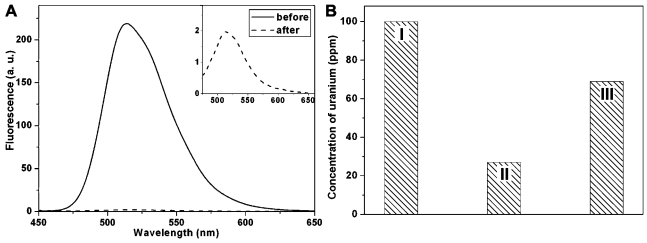

Figure 2. (A) Fluorescent spectra (λ_{ex} = 270 nm) of 1 mL solution of uranium nitrate before ($[UO_2^{2+}] = 10^{-4}$ M) and after ($[UO_2^{2+}] = 10^{-6}$ M) decorporation using 5 mg of **5**. (B) Inductively coupled plasma spectrometry (ICP) analysis result of the amounts of UO_2^{2+} in blood (I) before and (II) after the removal process, and (III) the amounts of UO_2^{2+} on the magnetic nanoparticles.

easily removed **6** from the solution. We used fluorescent spectra to assess the efficiency of removal since UO_2^{2+} gives emission at 513 nm upon excitation (λ_{ex} = 270 nm). The fluorescent spectra (Figure 2A) indicate that this simple procedure is able to remove 99% of UO_2^{2+} from the sample. In addition, the ToF-SIMS spectrum of **6** shows the fragments of iron-coordinated **4** with UO_2^{2+} ((FeO_2·**4** + UO_2)$^+$, *m*/*e* = 810, 826, 842) and a high intensity peak of UO_2^{2+} (UO_2^{2+}, *m*/*e* = 135), which agrees with the high affinity of **5** toward UO_2^{2+}. TEM of **6** (Figure 1C) shows the loss of lattice image, likely due to the high-electron-density uranium ions chelated by the bisphosphonate groups on the surface of **6**, which also agrees with the magnetic moments of **5** and **6**.[11] EDP (Figure 1D) reveals that the core of **6** remains as crystalline Fe_3O_4.

The above results prove the principle of the design shown in Scheme 1B and encourage us to test decorporation of UO_2^{2+} from blood. We added 15 mg of **5** into 1 mL of blood containing UO_2^{2+} (100 ppm) and sonicated the mixture to ensure the sufficient interaction between **5** and UO_2^{2+}. Then we used a small magnet to attract and remove the magnetic nanoparticles from the blood. To avoid the interference of auto-fluorescence of blood, we chose to use ICP to determine the amount of UO_2^{2+} left in the blood and absorbed onto the nanoparticles. As shown in Figure 2B, **5** removes 69% of UO_2^{2+} from the blood, with 4% lost during the experiment and 27% remaining in blood. Following the definition used in the MACS process, we also calculated the partitioning coefficient (K_d) for the removal of UO_2^{2+}. At pH = 7.0, the K_d values are 19 800 and 180 mL/g for removing uranyl ions from water and blood, respectively.

In summary, we successfully synthesized a bisphosphonate derivative, DA−BP, which modifies the magnetite nanoparticles to provide a very efficient protocol to remove uranyl ions from blood. Although the phosphates and proteins in the biological systems (e.g., blood) also bind to UO_2^{2+} competitively, the high affinity between the bisphosphonate and uranyl ions (due to chelating effect[12]) allows successful decorporation. This work provides a potential platform to develop biocompatible methodology (e.g., using magnetite nanoparticles and appropriate ligands) for decorporating radioactive hazards from the human body. In addition, the principle demonstrated in this work should also allow the detection, recovery, and decorporation of other heavy metal toxins from biological systems via tailoring the ligands or utilizing other novel nanomaterials.[13]

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Supporting Information Available: Detail of the syntheses, the calibration, the magnetic measurement, and the calculation. This material is available free of charge via the Internet at http://pub.acs.org.

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