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Functionalized Europium Oxide Nanoparticles Used as a Fluorescent Label in an Immunoassay for Atrazine

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A method for simply and cheaply preparing inorganic phosphor nanoparticles of Eu_2O_3 as labels in biology has been demonstrated with a simple microwave-assisted surface chemistry. The capping process adds a silane layer to the surface of the particles and provides amine groups that can be used for biological conjugation. The surface layer also protects the particles during conjugation chemistry. The particles retain their desirable optical properties that are typical of europium, that is, a spectrally narrow, red emission and a long fluorescence lifetime. The application of the nanoparticle labels in an immunoassay yields very good sensitivity in an immunoassay for atrazine (sub-parts-per-billion detection limit) without optimization of the detection system. The microwave functionalization technique will permit a broad range of inorganic nanophase phosphors to be used in high-throughput assays for environmental monitoring.

Fluorescent labeling of molecules is a standard technique in biology.[1] The labels are often organic dyes that give rise to the usual problems of broad spectral features, short lifetime, photobleaching, and potential toxicity to cells. Alternative labels may be based on lanthanide-derived phosphors. For example, the optical properties of europium chelates have been widely employed in molecular labels for the sensitive detection of proteins and nucleic acids,[2] in time-resolved fluorometric assays[3,4] and in immunoassays.[5−7] In the case of immunoassays, about 10 or more Eu^{3+} chelates can usually be incorporated into one antibody molecule without having a significant impact on the binding affinity of the antibody.[8]

Chelated lanthanide labels offer the potentially significant advantage of a relatively long-lived emission if quenching by water and dissolved oxygen can be avoided. In time-resolved (or time-gated detection) fluorometric assays with europium chelate labels, the background can be substantially reduced by detecting the signal after a time delay; in this way, the short-lived, nonspecific background interference is effectively reduced. For example, in a cofluorescence enhancement system, the lifetime of Eu^{3+} emission was 0.85 ms,[9] much longer than the typical background fluorescence of biological origin. Furthermore, labeling with fluorescent europium chelates is free of problems related to quenching.[10] The emission is also well-separated spectrally from the excitation wavelength and from the background emission that appears in environmental and biological samples. However, the chelation chemistry is often expensive and complex. As a result, their application has been limited.

Recently, nanoparticles, such as quantum dots, have attracted a great deal of attention in biology.[11−15] These particles can have a strong optical emission that exhibits a sharper spectral peak than typical organic dyes, with a large Stokes shift, and with minimal influence by other chemicals. In addition to semiconductor quantum dots, simple inorganic phosphors such as Eu_2O_3 have been recognized as offering potential in obtaining high emission intensity.[16,17] Unfortunately, natural or untreated Eu_2O_3 particles are insoluble in water and are easily dissolved by acid during activation and conjugation, losing their desirable optical properties.

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(1) Slavik, J. *Fluorescent Probes in Cellular and Molecular Biology*; CRC Press: Boca Raton, FL, 1994.

(2) Lim, M. J.; Patton, W. F.; Lopez, M. F.; Spofford, K. H.; Shojaee, N.; Shepro, D. *Anal. Biochem.* **1997**, *245*, 184−195.

(3) Liu, X. J.; Li, Y. Z.; Ci, Y. X. *Anal. Chim. Acta* **1997**, *345*, 213-217.

(4) Yuan, J. L.; Wang, G. L.; Majima, K.; Matsumoto, K. *Anal. Chem.* **2001**, *73*, 1869−1876.

(5) Scorilas, A.; Bjartell, A.; Lilja, H.; Moller, C.; Diamandis, E. P. *Clin. Chem.* **2000**, *46*, 1450−1455.

(6) Vaisanen, V.; Harma, H.; Lilja, H.; Bjartell, A. *Luminescence* **2000**, *15*, 389−397.

(7) Qin, Q. P.; Lovgren, T.; Pettersson, K. *Anal. Chem.* **2001**, *73*, 1521−1529.

(8) Xu, Y. Y.; Pettersson, K.; Blomberg, K.; Hemmila, I.; Mikola, H.; Lovgren, T. *Clin. Chem.* **1992**, *38*, 2038−2043.

(9) Diamandis, E. P. *Clin. Chem.* **1991**, *37*, 1486-1491.

(10) Diamandis, E. P. *Analyst* **1992**, *117*, 1879−1884.

(11) Casci, T. *Nat. Rev. Genet.* **2001**, *2*, 567.

(12) Chan, W. C. W.; Nie, S. M. *Science* **1998**, *281*, 2016−2018.

(13) Han, M. Y.; Gao, X. H.; Su, J. Z.; Nie, S. *Nat. Biotechnol.* **2001**, *19*, 631−635.

(14) Mattoussi, H.; Mauro, J. M.; Goldman, E. R.; Green, T. M.; Anderson, G. P.; Sundar, V. C.; Bawendi, M. G. *Phys. Status Solidi B* **2001**, *224*, 277−283.

(15) Rosenthal, S. J. *Nat. Biotechnol.* **2001**, *19*, 621−622.

(16) Nogami, M.; Abe, Y. *J. Non-Cryst. Solids* **1996**, *197*, 73−78.

(17) Patra, A.; Sominska, E.; Ramesh, S.; Koltypin, Y.; Zhong, Z.; Minti, H.; Reisfeld, R.; Gedanken, A. *J. Phys. Chem. B* **1999**, *103*, 3361−3365.

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Coating the particles can protect them from being dissolved by acid and may provide useful functional groups for biological conjugation.

Numerous materials are suitable for capping particles. Silica and alumina surfaces exhibit a useful range of surface reactivities.[18] In particular, silica can be used as a cap to keep europium oxide from dissolving in acid during the conjugation process. However, coating with silica and alumina may increase the particle size. Directly coating the particles with a Si-containing matrix may provide the best way to preserve the optical properties of Eu_2O_3 nanoparticles and to provide biologically functional groups at the same time. In this paper, we describe a new way for obtaining europium oxide labels that provide an amine group for conjugation and demonstrate their efficacy in a model immunoassay.

Atrazine, a member of the triazine herbicide family, was chosen as a test case for the evaluation of Eu_2O_3 particles in an immunoassay. Because these compounds have been the most heavily used agrochemicals in the world, triazine compounds appear frequently in groundwater.[19] The widespread use of atrazine, resulting in contaminated drinking water and food products, poses the risk of exposure for the general public[20] and for the environment.[21] Recent controversy surrounding the effects of atrazine on amphibians[22] emphasized the need for careful monitoring of this class of compounds.

The environmental significance of atrazine highlights the pressing need to carry out high-throughput, sensitive assays for members of the triazine herbicides. Environmental assays for atrazine must be performed in many different matrixes (i.e., soil, water, plants, urine), many of which contain compounds that fluoresce. Hence, our ultimate interest is in applying the Eu_2O_3 labels in an immunoassay for atrazine, taking advantage of both a large Stokes shift in the emission, the longer wavelength of the emission that may avoid reabsorption in the matrix, and long fluorescence lifetime of Eu_2O_3, that offers the possibility of using time-gated detection to avoid autofluorescence problems. We demonstrate that it is possible to use relatively cheap and widely available reagents to carry out the atrazine immunoassay using a europium label.

Europium nanoparticles have been used previously as labels for time-resolved bioassays. Harma et al.[23] entrapped chelated europium in polystyrene particles that had diameters ranging from ~100 to ~400 nm; carboxyl groups on the surface of the polymer particles were used in the conjugation to biomolecules. Although this scheme was employed successfully, we have devised a simpler method for using europium oxide nanoparticles directly, without the need for chelation or entrapment in another medium. The key to the successful application of a potentially wide range of inorganic materials in biology lies with microwave heating.

Microwave chemistry has been found to be particularly useful in exploiting novel chemical syntheses.[24,25] In particular, microwave absorption by lanthanides has been demonstrated and utilized in several applications.[26,27] We have found that 3-aminopropyltrimethoxysilane (APTMS) is microwave-transparent, whereas Eu_2O_3 absorbs microwave energy, arising from either an intrinsic absorption, or possibly as a result of water or OH attached to the particle surfaces. The differential absorption in the particle suspension leads to heating that can be confined to local regions without the need to heat the bulk solution. Reaction between the APTMS and the particle is concentrated at the particle surface. A reaction at the solid−liquid interface yields a layer of Si−O−$(CH)_x$−NH_2 that is covalently bound to the Eu_2O_3 particles.

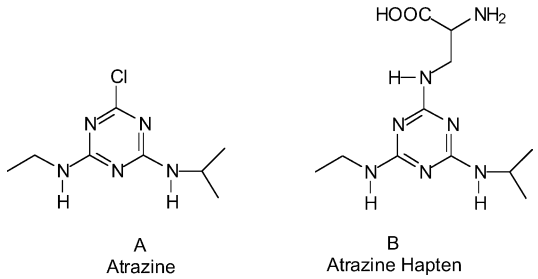

Figure 1. (A) and (B) atrazine and derivative used for conjugation.

EXPERIMENTAL SECTION

Chemicals. Commercial europium(III) oxide powder, ninhydrin, APTMS, 2-chloro-4-(ethylamino)-6-{(1-methylethyl)amino}-1,3,5-triazine (Figure 1A) and *N*,*N*-diisopropylethylamine were the products of Aldrich Chemical Co. The atrazine hapten (2-amino-3-(4-(ethylamino)-6-isopropylamino-(1,3,5)-triazin-2-ylamino) propionic acid; Figure 1B) was prepared in the manner described by Goodrow et al.[28] Magnetic beads coated with goat anti-mouse IgG (1 mg ml^{-1}) were purchased from Polysciences, Inc. (Warrington, PA). The monoclonal anti-atrazine antibody (AM7B.2) was provided by Dr. A. E. Karu (University of California, Berkeley) and has been described by Karu et al.[29]

Apparatus. Magnetic beads were separated from the solution with an Ohmicron 60-position magnetic rack (Strategic Diagnostics Inc., Newark, DE). Fluorescence measurements were performed in an ISA FluoroMax 2 fluorescence spectrophotometer (Jobin-Yvon, Inc., Edison, NJ). The microwave system that was used to prepare capped nanoparticles was a 1-kW basic domestic unit.

Functionalization of Particles. The Eu_2O_3 particles from Aldrich were suspended in ethanol and then sonicated in a bath for half an hour. The suspension was separated from the sediment (removing large particles) and then centrifuged at 6000*g* for 30 min. The resulting pellet was separated and dried. A 5-mg portion of the dried particles was added to a 10-mL beaker, and ~0.5 mL of undiluted APTMS was then added to cover the Eu_2O_3 particles.

(18) Horrocks, W. D.; Collier, W. E. *J. Am. Chem. Soc.* **1981**, *103*, 3, 2856−2862.
(19) Cohen, S. Z.; Eiden, C.; Lorber, M. N. *ACS Symp. Ser.* **1986**, *315*, 170.
(20) Loosli, R. *Rev. Environ. Contam. Toxicol.* **1995**, *143*, 47−57.
(21) Renner, R. *Environ. Sci. Technol.* **2002**, *36*, 55A−56A.
(22) Hayes, T.; Haston, K.; Tsui, M.; Hoang, A.; Haeffele, C.; Vonk, A. *Nature* **2002**, *419*, 895−896.
(23) Harma, H.; Soukka, T.; Lovgren, T. *Clin. Chem.* **2001**, *47*, 561−568.
(24) Kaiser, N. F. K.; Bremberg, U.; Larhed, M.; Moberg, C.; Hallberg, A. *Angew. Chemie* **2000**, *39*, 3596−3598.
(25) Usyantinsky, A. Y.; Khmelnitsky, Y. L. *Tetrehedron Lett.* **2000**, *41*, 5031−5034.
(26) Sen Gupta, J. G.; Bertrand, N. B. *Talanta* **1995**, *42*, 1595−1607.
(27) Haque, K. E. *Int. J. Miner. Process.* **1999**, *57*, 1−24.
(28) Goodrow, M. H.; Harrison, R. O.; Hammock, B. D. *J. Agric. Food Chem.* **1990**, *38*, 990−996.
(29) Karu, A. E.; Harrison, R. O.; Schmidt, D. J.; Clarkson, C. E.; Grassman, J.; Goodrow, M. H.; Lucas, A.; Hammock, B. D.; Van Emon, J. M.; White, R. J. In *Immunoassays for Trace Chemical Analysis: Monitoring Toxic Chemicals in Humans, Food, and Environment*; Vanderlaan, M., Stanker, L. H., Watkins, B. E., Roberts, D. W., Eds.; ACS Symposium Series 451; American Chemical Society: Washington, DC, 1991; pp 59−77.

Following sonication in a bath for 15 min, the mixture was put onto the rotating stage of the microwave oven and heated at full power for 2 min, followed by further stirring and sonication for 5 min. The heating and sonication procedure was repeated 10 times until the suspension became a sticky mixture. The material was maintained at room temperature overnight, then ground and heated at 150 °C for 30 min. The resulting particles were suspended in distilled water and centrifuged. The ensuing pellet was washed with distilled water three times and dried. Following this procedure, the particles were ready for conjugation to the atrazine hapten.

Particle Characterization. Samples of the Eu_2O_3 particles were examined by transmission electron microscope (TEM). The crystal structure was determined from X-ray diffraction measurements (XRD). The functionalized Eu_2O_3/SiO particles were characterized using two different approaches: (1) fluorescence spectrophotometry to measure the excitation spectrum (observed at 610 nm) and emission spectrum (excited at either 394 or 466 nm) of europium oxide particles and (2) a ninhydrin test [30,31] to detect the presence of $-NH_2$ groups on the particles and to quantitate the number of amine groups on the europium oxide particles by measuring the absorbance at 570 nm. For quantification of amine groups, a standard curve was created using glycine as a reference material.

Conjugation. The conjugation reaction used two stock solutions. Stock solution A: 18.4 mg of atrazine hapten was dissolved in 2.4 mL of $CHCl_3$ with one drop of *N*,*N*-dimethylformamide (DMF) in a 20-mL round-bottom flask at 0−4 °C, then 1.5 mL of thionyl chloride ($SOCl_2$) was added dropwise over 5 min. The mixture was stirred and heated to between 65 and 75 °C for 1 h. The flask was put on a rotary evaporator to strip the solvent and excess thionyl chloride. Stock solution B: 5 mg of the activated Eu_2O_3 particles was suspended in 2 mL DMF and added to 15 mg of *N*,*N*-diisopropylethylamine. Solutions A and B were mixed in a round-bottom flask at −20 °C. The temperature was maintained by use of an iced sodium chloride water bath; the mixture was stirred overnight, and the water bath temperature was gradually increased to room temperature.

Atrazine ELISA. The application of the functionalized nanoparticles in a competitive immunoassay for atrazine is presented schematically in Figure 2. Atrazine herbicide and the atrazine-particle conjugates were suspended in a phosphate buffer solution (PBS: 8 g L^{-1} of NaCl, 1.15 g L^{-1} of Na_2HPO_4, 0.2 g L^{-1} of KCl, and distilled water). Monoclonal anti-atrazine antibody AM7B.2 solutions were diluted in PBSB (PBS containing 0.2% of BSA). Aliquots (100 μL) of atrazine particles (25 μg mL^{-1}, particles by weight), of AM7B.2 (0.1 nmol, delivered in 100 μL), and of different dilutions (∼0.5 to 25 ng/mL) of atrazine (delivered in 100 μL) were mixed in 12 × 75-mm borosilicate glass test tubes containing 600 μL of PBS and incubated at 30 °C for 30 min. Then 100 μL of magnetic beads coated with goat anti-mouse IgG was added and incubated at room temperature for 30 min with shaking. The beads were separated from the solution by placing the tubes onto the Ohmicron magnetic rack. The beads were washed with 1 mL of PBS, after which the fluorescence intensities in the pooled solutions (total 2 mL) were measured using the fluorescence spectrophotometer with excitation at 394 nm and emission at 610 nm.

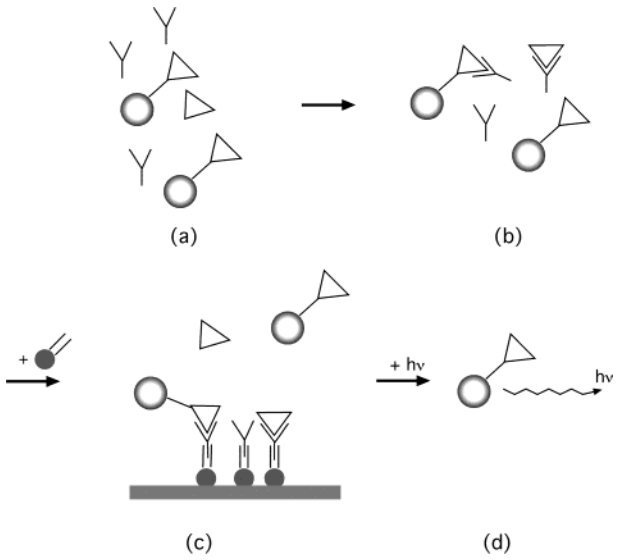

Figure 2. Schematic presentation of the competitive assay using magnetic separation. The assay was carried out as follows: (a) the atrazine-derivatized europium particles (O-∇), the free atrazine in the sample (∇), and the monoclonal anti-atrazine antibody (Y) are added together and (b) allowed to react. (c) Magnetic beads coated with anti-mouse antibody are then added. Free antibody, antibody bound to free analyte (∇−Y), and antibody bound to the atrazine-derivatized europium particles are all removed from the solution, leaving only atrazine-derivatized europium particles in solution. (d) The solution is then measured for fluorescence. If the analyte concentration is low, then most of the atrazine-derivatized europium particles will be bound to anti-atrazine antibody and subsequently to the magnetic beads and removed from solution; thus, the fluorescence of the solution will be low. If the analyte concentration is high, then the anti-atrazine antibody will be bound to the free analyte. When the magnetic beads are applied, very little atrazine-derived antibody will be removed from solution, since most of the antibody is bound to free atrazine; thus, the fluorescence of the solution will be high. This format results in a calibration curve in which the concentration of free analyte in the sample is directly proportional to the amount of fluorescence detected in the solution.

RESULTS

The untreated commercial Eu_2O_3 particles from Aldrich exhibited polydisperse primary particle sizes from ∼20 to 100 nm, determined by TEM (lower image of Figure 3). The primary particles were aggregated into polydisperse clusters of 300−600 nm (upper image of Figure 3). Examination of the particles with XRD indicated that the crystalline material was cubic Eu_2O_3.

The Eu_2O_3 particles have a useful excitation region from ∼356 to 410 nm, with a maximum at 394 nm. Another strong absorption is located at 466 nm. Following excitation at either 394 or 466 nm, the Eu_2O_3 particles produced an emission feature at 610 nm (Figure 4). The emission spectrum has the following salient characteristics, typical of europium and its chelates: (1) large Stokes shift (144 or 216 nm, depending on excitation wavelength); (2) a narrow, symmetric emission feature at around 610 nm (full width half-maximum of 8 nm, corresponding to the $^5D_0 \rightarrow {}^7F_2$ transition); and (3) a long lifetime (in this case, measured with a time-resolved fluorescence system to be ∼100 μs). Most impor-

(30) Henry, A. C.; Tutt, T. J.; Galloway, M.; Davidson, Y. Y.; McWhorter, C. S.; Soper, S. A.; McCarley, R. L. *Anal. Chem.* **2000**, *72*, 5331−5337.
(31) Taylor, I.; Howard, A. G. *Anal. Chim. Acta* **1993**, *271*, 77−82.

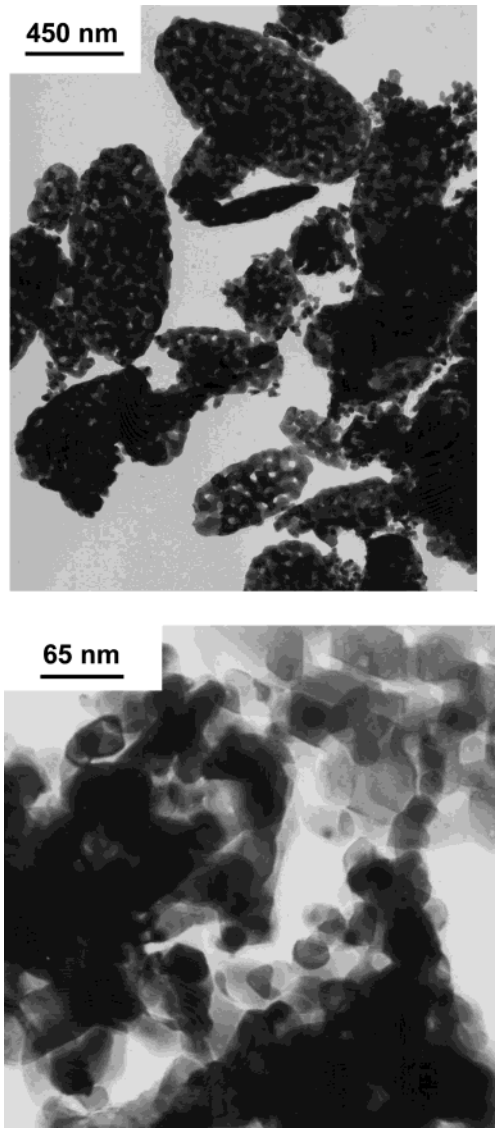

Figure 3. Transmission electron microscope images of Eu_2O_3 particles.

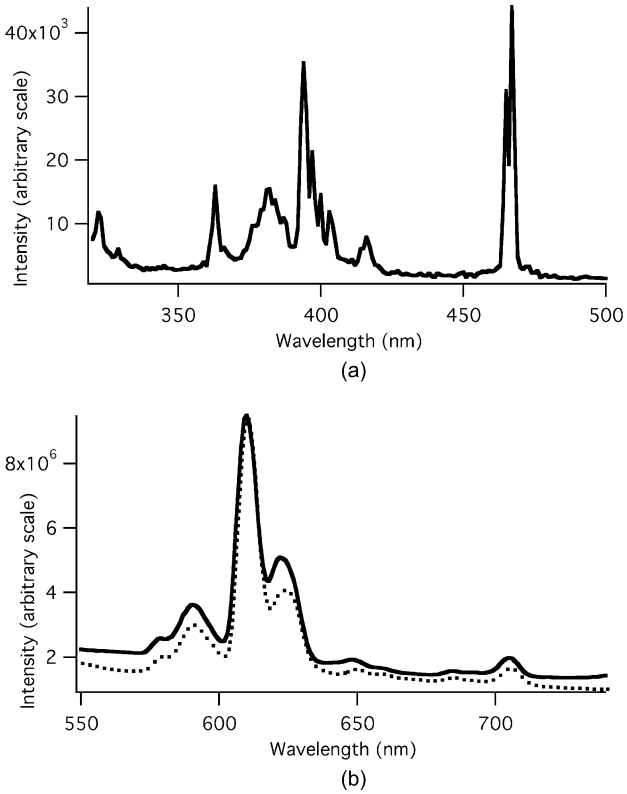

Figure 4. Fluorescence spectra of the functionalized Eu_2O_3 particles. (a) Excitation spectrum observed at 610 nm; (b) fluorescence emission spectrum with excitation at 466 nm (dashed curve shows emission from uncapped Eu_2O_3 particles).

tantly, these characteristics were unchanged by the functionalization process. The strong absorption at 466 nm is not commonly reported for Eu materials, although Lima et al.[32] noted a strong excitation of Eu in ZnO at 465 nm; they attributed the absorption to the $^5D_0 \leftarrow {}^7F_0$ transition in Eu. Its presence has been confirmed on two separate instruments. It may prove to be useful in avoiding ultraviolet excitation of background signals of biological origin.

A narrow emission band from a fluorophore is potentially quite advantageous in bioanalysis. Considerable attention has been given to novel fluorophore labels in recent years, with a view to exploiting narrow spectral lines. For example, nanoscale quantum dots have an emission that is much narrower than conventional organic dyes. A typical functionalized, water-soluble, nanocrystal of CdSe/ZnS in PBS was excited at 355 nm, leading to an emission at 533 nm with a 32-nm fwhm.[33] In comparison, the Eu_2O_3 nanoparticles exhibit a considerably narrower emission that will permit very selective detection against a large, spectrally broad background.

Microwave-assisted coating with APTMS proved to be simple but effective. The structure of the surface layer is likely to be similar to those proposed by Gerion et al.,[34] who developed a complex wet chemical method for functionalizing CdSe/ZnS quantum dots with a similar capping layer. The coverage of $-NH_2$ was measured in our case to be ~10 nmol mg^{-1}. Some of the aggregation seen in Figure 3 may be due to the surface layer on the particles; alternative capping chemistries are being explored to minimize aggregation.

The potential of coated Eu_2O_3 fluorescent nanoparticles as a label for biological application was demonstrated with atrazine hapten−Eu_2O_3 particle conjugates in a competitive immunoassay for the herbicide atrazine. Figure 5 shows the fluorescence intensity of unbound Eu−atrazine conjugates in solution measured at 610 nm. The detection limit of this assay is ~0.5 ng mL^{-1}. This compares with a limit of detection of ~0.1 ng mL^{-1} in a conventional enzyme-linked immunosorbent assay (ELISA).[35] The sensitivity of the rapid europium-based assay is sufficient to resolve the near-parts-per-billion (ppb) levels at which Hayes et al.[22] reported effects on the gonadal development of amphibians. The conditions for the Eu_2O_3-based assay were the same as those used in the ELISA and were not optimized in any way. By employing this simple europium nanoparticle label in an assay for atrazine,

(32) Lima, S. A. M.; Sigoli, F. A.; Davolos, M. R.; Jafelicci, M. *J. Alloys Comps.* **2002**, *344*, 280−284.
(33) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016.
(34) Gerion, D.; Pinaud, F.; Williams, S. C.; Parak, W. J.; Zanchet, D.; Weiss, S.; Alivisatos, A. P. *J. Phys. Chem. B* **2001**, *105*, 8861−8871.
(35) Wortberg, M.; Kreissig, S. B.; Jones, G.; Rocke, D. M.; Hammock, B. D. *Anal. Chim. Acta* **1995**, *304*, 339−352.

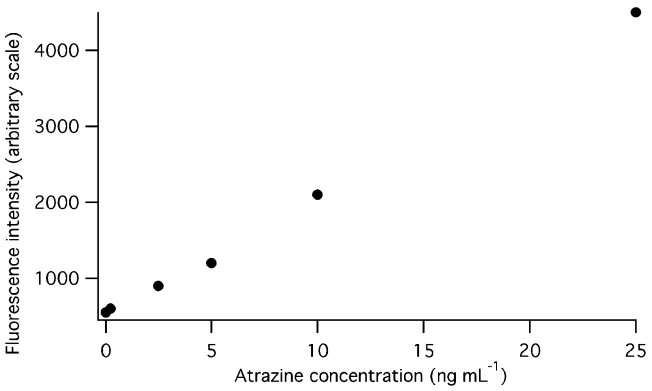

Figure 5. Competitive fluoroimmunoassay of atrazine using Eu_2O_3 particles as reporters. The background signal was subtracted from each data point. The assay was replicated three times with a standard deviation that was <5% of the reported values.

two steps in the standard ELISA were eliminated, resulting in a higher-throughput assay and a smaller coefficient of variation. The use of conventional organic fluorophores in the same assay increased speed but reduced total signal-to-noise, as compared to the classical ELISA, as a result of an increase in background (data not shown).

An interesting feature of the lanthanides needs to be noted at this point. Many of the optically useful lanthanides also exhibit paramagnetism. For this reason, an assay of this type has to be carried out in glass tubes and not in plastic tubes. In the latter case, europium oxide particles bind nonspecifically to the magnetic particles, rendering the immunochemical separation unworkable.

The comparison of sensitivities is encouraging and is indicative of the potential for the simply funtionalized inorganic Eu nanoparticles to improve practical detection methodologies in environmental toxicology. In addition to the useful optical properties (sharp spectral features, long lifetime, no photobleaching), the treated particles were found to be very easily purified and conjugated to proteins or haptens. Other areas of biology may benefit from the ease of use of simply prepared functionalized Eu_2O_3 particles. In addition, more complex crystal compositions with Eu doped into Al_2O_3, Y_2O_3, or Gd_2O_3 hosts may offer additional wavelengths for use in multiplexed bioassays, as well as providing greater quantum efficiency by avoiding the concentration quenching that is characteristic of the pure oxide. Other members of the lanthanide family can be treated in the same manner and can offer a wide range of optical emissions, in addition to magnetic properties that may also be quite useful for separations.

Unlike quantum confined semiconductor particles, the optical emission from the functionalized lanthanide nanoparticles does not rely sensitively on an intrinsic property that arises from their size, although some dependence on surface states has been shown in nanocrystals of Eu_2O_3.[36,37] On the other hand, the lanthanide oxide nanoparticles that we used are readily available in bulk and are easier to functionalize than quantum dots. However, the particles are relatively large (compared to quantum dots) and are polydisperse.

Clearly, larger particles emit more light and would serve to improve signal-to-noise ratios in bioassays. In addition, the fluorescence decay time is longer in larger particles,[37] aiding in gated detection schemes. However, at some point, large particles are likely to interfere with the antibody binding reactions. In addition, diffusion can be a rate-limiting process in microfluidic systems: large particles will reduce the rates of mixing of reagents by diffusion. An optimum particle size may exist that yields high sensitivity without the undesirable effects that have been noted. The impact of size on the optical properties of europium oxide particles and on the efficacy of their application in fluoroimmunoassays is under investigation.

The commercial material that was used in this preliminary investigation exhibited a broad size distribution. The size distribution was also affected to some extent by aggregation that may have resulted from the nature of the functionalization. This may pose problems for assays in small volumes in which the number of labels is small and the variance in particle size may contribute to uncertainty in the result. In the present experiments, a statistically large number of particles were always interrogated so that size distribution issues were not dominant. However, a more nearly monodisperse collection of particles would be desirable and could be obtained by a fractionation step with greater finesse than the centrifuging used in these experiments. Alternative capping processes are also being explored with a view to minimizing the impact of aggregation.

CONCLUSION

A simple and rapid method has been devised and demonstrated for the functionalization of inorganic metal oxide nanoparticles that are commercially available. The very attractive fluorescence properties of europium are preserved in this process, offering labels for biology that exhibit long fluorescence lifetime and sharp spectral features. The initial application of this material to an immunoassay for atrazine showed promise as a simplified competitive assay with little reduction sensitivity.

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(36) Eilers, H.; Tissue, B. M. *Chem. Phys. Lett.* **1996**, *251*, 74−78.
(37) Bihari, B.; Eilers, H.; Tissue, B. M. *J. Luminesc.* **1997**, *75*, 1−10.