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Sequestration, Fluorometric Detection, And Mass Spectroscopy Analysis of Lanthanide Ions Using Surface Modified Magnetic Microspheres for Microfluidic Manipulation

Ilya A. Shkrob,*,† Michael D. Kaminski,*,† Carol J. Mertz,† Paul G. Rickert,† Mark S. Derzon,‡ and Kamyar Rahimian§

Chemical Sciences and Engineering Division, Argonne National Laboratory, 9700 South Cass Ave, Argonne, Illinois 60439 and MEMS Technologies and Organic Materials Departments, Sandia National Laboratories, Albuquerque, New Mexico 87185

Received April 30, 2009; E-mail: shkrob@anl.gov

Abstract: Several methods for rapid sequestration, fluorometric detection, and the subsequent mass spectroscopic analysis of lanthanide ions using surface modified polystyrene magnetic microspheres are demonstrated. Mixed-ligand antenna complexes of Eu^{3+} in which one of the ligands is attached to the surface of the microspheres have been used as a means for the sequestration, immobilization, and detection of these ions. Using the ion-exchange properties of these microspheres, this scheme has been extended to the detection of nonluminescent ions. The principles of these assays form the basis for operation of a portable microfluidic device for general analytical and nuclear forensics applications and indicate the manner in which the established methods of analytical chemistry, such as liquid−liquid extraction and ion-exchange chromatography, can be adapted for such miniature devices.

Introduction

Liquid−liquid extraction and ion-exchange chromatography are common methods for preconcentration and separation of metal ions for subsequent trace element analyses, but these methods are unsuitable for microfluidic miniaturization of analytical procedures.[1] Surface modified magnetic microspheres can replace these methods provided that new techniques for sequestration and detection of the metal ions on these magnetic microspheres are demonstrated.[2,3] Ideally, the sequestration should provide the means for transporting the selected ion through the microfluidic device followed by sensitive and nondestructive detection. In the present work, several schemes for sequestration and detection of lanthanide ions using the magnetic microspheres are reported. These magnetic microspheres have been used as a versatile platform for immobilization of trace amount (<1 ppb) of fission products that are subsequently detected using time-resolved laser fluorescence (TRLF) spectroscopy, and their isotopic composition is determined using mass spectrometry. These assays serve as the basis for operation of a portable microfluidic device for nuclear forensics that is developed at Argonne and Sandia National Laboratories. To shorten this work, the details of the assays, the methods, and the synthetic and conjugation protocols are given in the Supporting Information. While Eu^{3+} was chosen to demonstrate the methods, the approach is not limited to the luminescent ions.

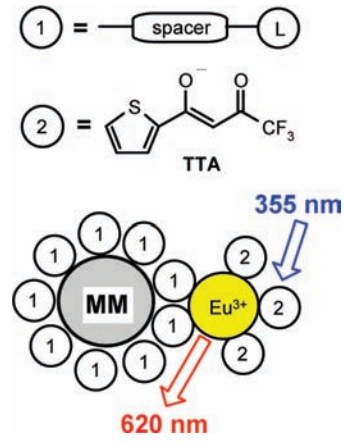

Figure 1. Conceptual scheme of a mixed ligand antenna assay for sequestration and TRLF detection of Eu^{3+} ions.

† Chemical Sciences and Engineering Division, Argonne National Laboratory.
‡ MEMS Technologies, Sandia National Laboratories.
§ Organic Materials Departments, Sandia National Laboratories.

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Table 1. Properties of "Smooth" Polystyrene Magnetic Microspheres Supplied by Spherotech[a]

type	mean diameter, μm	area,[b] $\times 10^7$ nm^2	surface group	surface concentration of groups, per nm^2	total number of groups,[b] $\times 10^8$	volume concentration, $\times 10^9$ cm^{-3}[a]	molar concentration of groups, μeq/L[a]
PMS-20	2.5	2.0				2.8	
CMS-30	4.7	3.4	CO_2	4.2	1.4	1.24	290
AMS-40	3.3	7.0	NH_2	1.7	1.8	0.42	80

[a] For a 2.5 wt % stock solution. [b] Per microsphere.

The general approach (Figure 1) is similar to the well-known DELFIA (dissociation enhanced lanthanide fluorescence) immunoassay,[4,5] in which Eu^{3+} forms a luminescent complex, $Eu^{III}L_2A_3$, where L is a neutral ligand, such as trin-octylphosphine oxide (**1**) and A is an antenna ligand, such as the base of 2-thenoyltrifluoroacetone (TTA, p$K_a \approx 6.32$; Figure 1). The antenna ligand absorbs 355 nm laser light and the excitation energy is transferred to Eu^{3+} that emits at 620 nm via $^7F_2 \leftarrow {}^5D_0$ transition, with the emission lifetime of 0.5−0.75 ms.[5] This long-lived luminescence allows background-free detection of as little as 1 ppt of Eu^{3+} by means of TRLF, as it discriminates against the short-lived fluorescence from the organic molecules.[4] In our scheme, the neutral ligand is attached to the surface of the microspheres. Because water molecules in the coordination sphere of Eu^{3+} quench the luminescence, the complex must be isolated in a hydrophobic environment in order to inhibit ligand exchange with the aqueous solution. One of the challenges of adapting the extraction protocols to the magnetic microsphere platform is that the hydrophobic ligands at the surface cause aggregation of the modified microspheres, which is undesirable for microfluidic manipulation. In order to keep the microspheres fully suspended, these must be covered by nonionic detergents. Since such surfactant molecules tend to form micelles in the solution that extract hydrophobic Eu^{3+} complexes, the system has to be carefully designed to bias the extraction of the complex to the hydrophobic surface of the magnetic microspheres.

Another challenge is quenching of the luminescence by the magnetic microspheres. Since it interferes with the TRLF detection, it was necessary to search for the magnetic microspheres that do not quench the luminescence on the submillisecond time scale. All but one brand of commercially available magnetic microspheres were shown to be efficient luminescence quenchers on this time scale due to the presence of dispersed ferric ions near the surface that serve as the sinks for the excitation energy. These ferric ions are residues from the incorporation of magnetic nanoparticles into the magnetic microspheres during their synthesis. Spherotech Inc. supplies polystyrene magnetic microspheres that have a 1-μm protective overcoat of polystyrene that isolates these dispersed ferric ions, and only such "smooth" magnetic microspheres were shown to support long-lived luminescence from the *f*-elements. These carboxylated and aminated magnetic microspheres also have sulfonate groups at the surface that can be used for ion exchange. The relevant properties of these magnetic microspheres are given in Table 1. To prevent the aggregation of these surface modified microspheres, 0.1% decanoyl-*N*-methylglucamide (MEGA10) was used as a detergent. The choice of this surfactant was dictated by the unusually high (for a nonionic detergent) critical micelle concentration (cmc) of 0.25 wt %. This permitted full coverage of the magnetic microspheres by the nonionic detergent without introducing the micelles in the bulk of the solvent, so the only hydrophobic environment for the extraction of the luminescent complex is provided by the detergent-covered surface of the microspheres. These MEGA10-stabilized polystyrene magnetic microspheres were fully suspendible and stable for months.

The experimental details, synthetic procedure, and assay protocols are given in section 1S of the Supporting Information.

Results

1. DELFIA-Like Volume Assays. In the DELFIA assay,[4] the luminescent complex of Eu^{3+} is formed inside the core of a nonionic micelle; the hydrophobicity of the core prevents rapid quenching of the luminescence by coordinated aqua ligand. The same approach can be used for sequestration and detection of Eu^{3+} using the magnetic microspheres. In the simplest approach, $10^{-13}-10^{-6}$ M Eu^{3+} ions (10^{-12} M = 1.5 ppt) were extracted from 1 mL of 10^{-4} M HNO_3 solution by covalently attached aminocarboxylic acids on $\sim 10^7$ microspheres. The signal was linear with Eu^{3+} concentration in the entire concentration range. The best results were obtained using iminodiacetic acid (IDA) modified BcMag-IDA silica magnetic microspheres from Bio-Clone Inc. (1 μm) and ethylenediaminetetraacetic acid (EDTA) modified MagaCell-EDTA cellulose magnetic microspheres (Cortex Biochem, 10 μm). For the former microspheres, partitioning coefficients up to 2.3×10^4 for the extraction of Eu^{3+} were obtained. The Eu^{3+}-loaded silica microspheres exhibited a sorption capacity of $\sim$0.65 μeq per g of the microspheres (0.14 ions per nm^2) with a binding constant $\beta \approx 4 \times 10^7$ M^{-1}.

These microspheres were magnetically separated and the luminescence was developed by addition of 0.1% Triton X-100 solution containing 0.5 mM TTA and a neutral coligand. The luminescent complex was extracted into the micellar phase, and the detection of 10^{-12} M of Eu^{3+} was demonstrated in this fashion. The coligand that was most efficient in luminescence enhancement was octyl(phenyl)-*N,N*′-di(*iso*-butyl) carbamoyl methyl phosphine oxide (**2**). Good results were also obtained using **1** and triphenylphosphine oxide. Other polyethylene glycol terminated nonionic detergents (RTX-100, Igepal CO520, Brij 35 and 36T, Triton X series detergents from 207 to 705) and cationic detergents (such as cetyl triethylamine bromide) yielded comparable results,[6,7] with the luminescence enhancement reaching maximum right above the cmc. The nonionic detergents based on carbohydrate derivatives, such as dodecyl-β-D-maltoside, exhibit such strong affinity for the phosphine oxides that

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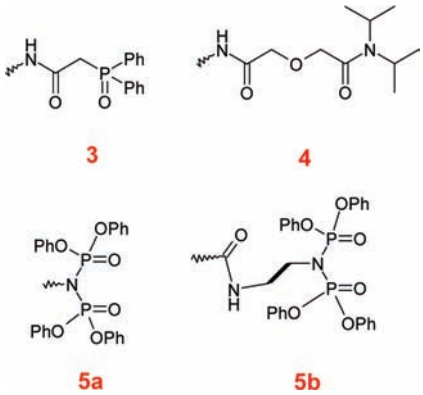
Scheme 1. Covalently Attached Neutral Ligands for Eu^{3+} Extraction at the Microsphere Surface



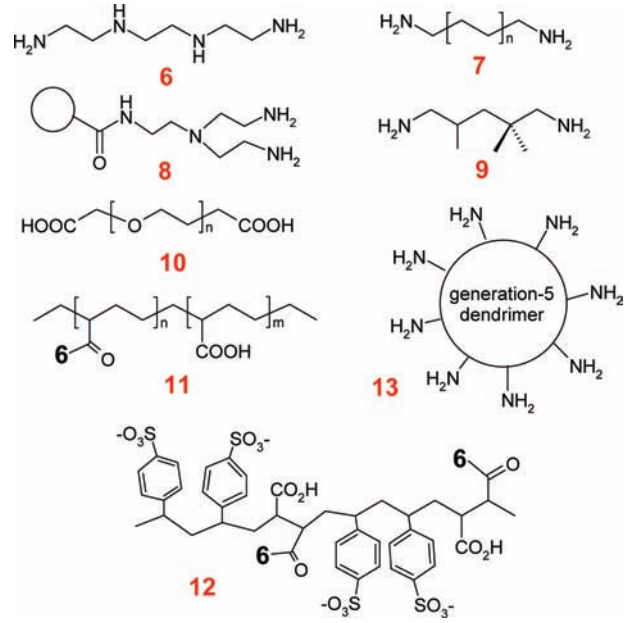
even water-soluble trimethyl and triethyl phosphine oxides form the luminescent complexes in their interior. The typical luminescence times were 650−720 μs. The sensitivity of the method can be improved further by increasing the volume of the sample and the contact time.

2. Surface Mixed-Ligand Complex Assays. The detriment of the DELFIA-like volume assay is that Eu^{3+} needs be stripped off the microsphere for TRLF and mass spectrometric detection, whereas it is preferable that the ion remains bound to the magnetic microspheres for the ease of microfluidic manipulation. This immobilization can be achieved either by physisorption and/or covalent binding of the ligands. In these assays, $(1-3) \times 10^7$ cm^{-3} of the "smooth" polystyrene magnetic microspheres were used to sequester the Eu^{3+} ions in 10^{-4} M HNO_3. All of the systems reported below exhibited extraction efficiencies of 95+% for pH 4−7 and $[Eu^{3+}] < 10^{-5}$ M, but the extraction efficiency decreased dramatically at pH < 3. This pH dependence allows stripping of Eu^{3+} from the magnetic microsphere by addition of 0.01−1 M HNO_3. Conversely, Eu^{3+} can be removed into the aqueous phase by addition of a strong water-soluble ion-binding complexant, such as $10^{-4}-10^{-3}$ M disodium salt of EDTA.

"Smooth" magnetic microspheres (Spherotech, model PMS-20, 2.3 μm) were impregnated by **2**, **1**, or their 1:2 mixture with tributyl phosphate in 20% NaCl solution containing 0.01% MEGA10 (~2.7 ligands per nm^2), which is equivalent to a monolayer coverage. The extracted luminescent complex was in a highly hydrophobic environment, as the luminescence lifetime was 350−430 μs. The magnetic microspheres were fully suspendible in these saline solutions. These microspheres extracted Eu^{3+} very efficiently and produced luminescence within 15 s after TTA was introduced into the analyte. The luminescence persisted after several cycles of magnetic separation followed by suspension of the magnetic microspheres in 0.1% MEGA10.

Alternatively, the extracting agents (Scheme 1) were covalently attached to the magnetic microsphere using the amide conjugation protocol. The general structure was microsphere surface-spacer-$(L)_n$, (Figure 1) where we used polyamines, polyethylene glycols, polyamidoamide dendrimers, and polymers as spacers (Scheme 2) to which one or several ligands were attached. The ligands were **2**-like moieties (**3**),[3,8,9] diglycolamide moieties (**4**),[10] and bidentate imide moieties (**5a** and **5b**) shown in Scheme 1. The simplest of these surface modifications involved reacting the aminated microspheres with $(PhO)_2P(O)Cl$ in dimethyl formamide; the detailed synthetic and conjugation protocols are given in section 1S, Parts D and E,

Scheme 2. Spacers for Neutral Ligands Shown in Scheme 1



of the Supporting Information, respectively. Complexation to TTA was used to develop the luminescence of the complex in less than 1 min. Most of these luminescent complexes have relatively short luminescence lifetime of 180 to 400 μs (Table 2), as these complexes include 1−2 water ligands (see below). However, these relatively short lifetimes are still sufficiently long to allow background-free detection of Eu^{3+} using TRLF. The sensitivity limit was $\approx 5 \times 10^{-11}$ M, and the linearity of the luminescence signal persisted over 5−6 decades in the Eu^{3+} concentration (Figure 2). For ligand **5a** directly attached to the surface, the sorption capacity for Eu^{3+} was ~50 μeq per g of the microspheres and the stability constant β of the complex was $\sim 1.4 \times 10^7$ M^{-1}.

In a typical assay, 300 μL of Eu^{3+} solution in 10^{-4} M nitric acid was mixed with 300 μL of 1−3 mM TTA and then 30 μL of 2.5 wt % of the modified magnetic microspheres were added, and the mixture was gently stirred for 30 s. The luminescence was measured at intervals of 30 s, with periodic agitation of the solution. The luminescence was fully developed in 1−2 min and then gradually subsided to 50−60% of the initial value due to the slow aggregation of the magnetic microspheres. The magnetic microsphere solution was magnetized and the lumi-

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Table 2. Relative Luminescence Yield in a Series of Trials[a] for Surface Modified Magnetic Microspheres

magnetic microsphere	spacer	ligand	TRLF signal[b]	lifetime, μs
AMS-40		**5a**	45	200
AMS-40		**3**	63	220
CMS-30		**5b**	91	280
AMS-30		**4**	35	220
CMS-30	**8**	**5a**	25	213
CMS-30	**8**	**3**	80	275
	8	**4**	27	250
CMS-30	**9**	**4**	26	240
AMS-40	**10**[c]	**5b**	73	240
AMS-40	**12**	**4**	95	260
CMS-30	**13**	**3**	43	280
CMS-30	**13**	**4**	36	270
PMS-20/**1**			100	310
PMS-20/**2**			71	270

[a] The conditions of the trials: 150 nM Eu^{3+} and 1.5 mM TTA in 600 μL of 5×10^{-5} M HNO_3 with 0.12 wt % of the surface modified microspheres. [b] Arbitrary units. [c] $n = 6$.

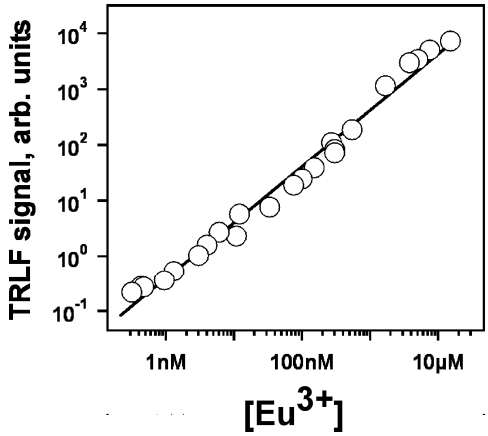

Figure 2. The concentration plot for TRLF signal for a magnetic microsphere assay using AMS-40-**5a** microspheres. Linearity of TRLF detection for magnetic extraction of Eu^{3+} by AMS-40 microspheres modified by **5a** in the presence of TTA.

nescence of the supernatant was measured to estimate the background contribution from the bulk of the solvent (which was typically negligible, < 1%). At the lowest concentration, 30 μL of 10 mM disodium salt of EDTA was added to the solution following the measurement in order to quench the luminescence and acquire the background signal. This residual signal was subtracted from the TRLF signal. The magnetically separated microspheres were suspended in 0.6 mL of 0.5−1.5 mM TTA in 0.1% MEGA10 or a 1:1 mixture of 0.1% MEGA10 and 10% NaCl. For some of the designs, the magnetization/resuspension cycle can be repeated 5−10× before the luminescence yield begins to decrease. For most of the designs, 2−3 of such cycles did not decrease the TRLF signal by more than 50%. While TTA enhances the surface binding of the Eu^{3+} ions, the presence of the coligand is not necessary during the sequestration. To demonstrate that, the Eu^{3+} ions were first extracted by 30 μL of 2.5 wt % microspheres from 300 μL of 10^{-4} M nitric acid solution; the microspheres were magnetically separated and then suspended in 600 μL of 0.5 mM TTA in 0.1% MEGA10 (or 10% NaCl, for "physisorption" assays). This pre-extraction method yielded luminescence that was similar or even higher than described above.

At pH < 3, the extraction efficiency of the surface-modified microspheres rapidly decreases with acidity, and at pH = 0 this efficiency is only 1−3%, even for the chelators (e.g., diglycolamides) that are known to be efficient extracting agents in ion-exchange resins in this pH range.[11,12] Clustering of such chelators failed to improve the extraction efficiency significantly, as was also observed by others.[3] Due to the high sensitivity of TRLF, reliable detection is still possible even at this high acidity, following the pH adjustment by tris(hydroxymethyl) aminomethane. The presence of 1 M NO_3^- in the solution reduces the luminescence yield ~3×, due to luminescence quenching by the nitrate anion. Using another method, the Eu^{3+} ions were extracted from 1 M HNO_3, the magnetic microspheres were separated, washed with 0.1 M imidazole buffer (pH 6) and then the luminescence was developed by addition of TTA in 0.1% MEGA10. This protocol resulted in poorer detectability limits, but it was still possible to detect 10^{-8} M of Eu^{3+}. The details of these low-pH adapted protocols are given in section 1S, Part F, of the Supporting Information.

Following the sequestration and the TRLF detection, the isotope composition for the metal ion can be detected using mass spectrometry, which is essential, inter alia, for obtaining forensics information from the samples. Three mass spectrometry methods have been demonstrated. Using the first method, 5−50 ppb of sequestered Eu^{3+} ions ($(0.3-3) \times 10^{-7}$ M) were stripped off the magnetic microspheres using 1 M HNO_3 and the supernatant was analyzed using inductively coupled plasma mass spectrometry. For the DELFIA-like solution assay, quadrupole electrospray ionization mass spectrometry was used to detect the $Eu(TTA)_2(\mathbf{2})_2^+$ complex in the gas phase, with the detection limit of 100 ppb. The $^{151}Eu/^{153}Eu$ isotope ratio was determined with the accuracy of 0.1% using both of these methods in the indicated concentration ranges. Alternatively, we used laser desorption/ionization mass spectrometry. Focused 337 nm laser light was used to photoexcite the surface of the microspheres, which obviated the need of acid stripping. The laser excitation of the magnetically separated microspheres resulted in the direct release of Eu^{3+} ions into the gas phase. The detection limit of ~0.1 ppb has been achieved, with 0.1% accuracy of isotope ratios at 50 ppb. The details of these mass spectrometry measurements are given in section 1S, Part G, Supporting Information.

3. Ion-Exchange Surface Assay. While the TRLF approach allows detection of luminescent *f*-ions, such as Eu^{3+} and Tb^{3+}, ion exchange properties of the microspheres can be used to detect *nonluminescent* ions at the concentrations that are comparable to the loading capacity of the microspheres. The microspheres considerably differ in their capacity as ion exchangers, and this capacity can be further tuned by changing the ionic strength. For the standard 0.25 wt % solution of the magnetic microspheres in 10^{-4} M HNO_3 containing 150 nM Eu^{3+}, the extraction efficiency of unmodified PMS-20, AMS-40, and CMS-30 microspheres is 90%, 70%, and 98%. In the identical solution containing 10% NaCl, this extraction efficiency is reduced to 43%, 0%, and 17%, respectively. The maximum capacity of PMS-20 microspheres for Eu^{3+} in these solutions was estimated at 0.24 mM. Other brands of the microspheres also demonstrated high capacity at low ionicity, but only functionalized microspheres, such as MagaCell-EDTA (Cortex) and ligand-conjugated AMS-40 and CMS-30 microspheres, exhibited high extraction efficiency at high ionicity. For good reproducibility, we found it necessary to have nonionic detergent

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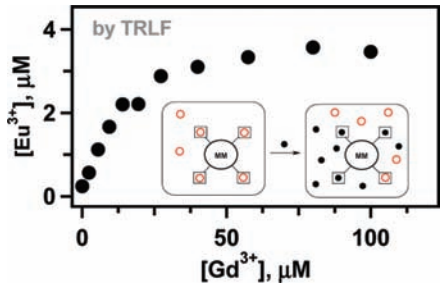

Figure 3. The release of Eu^{3+} from preloaded magnetic microspheres upon the addition of Gd^{3+}. The Eu^{3+} ions were detected using TTA/**2** in Triton X-100 micelles. The release of Eu^{3+} from preloaded CMS-30 microspheres upon the addition of Gd^{3+}. The Eu^{3+} ions were detected using TRLF in Triton X-100 micelles.

in the solution, as the aggregation properties of the magnetic microspheres changed as the latter were loaded with metal ions.

In the implementation of ion-exchange assay shown in Figure 3, 250 μL of the 0.07% suspension of CMS-30 carboxylated magnetic microspheres in 10^{-4} M HNO_3 also containing 0.1% Triton X-100 were equilibrated with 4.3 μM Eu^{3+} and then mixed with 250 μL of Gd^{3+} in 10^{-4} M HNO_3. After 1 min stirring, the solution was magnetized and 50 μL of the supernatant was mixed with 1.5 mL of 0.5 mM TTA and 0.5 mM **2** in 0.1% Triton X-100. The luminescence of Eu^{3+} was determined in this micellar solution and compared to the luminescence of the standard Eu^{3+} solution. Due to the great excess of the ligands in the micellar solution, the competition between Eu^{3+} and Gd^{3+} for these ligands was negligible. As seen from Figure 3, the luminescence signal is linear with $[Gd^{3+}]$ to 30 μM, though there is a weak luminescence background from free Eu^{3+} ions in the solution without Gd^{3+} ions, due to the extraction equilibrium (discussed below).

This assay can be carried out using TRLF detection of Eu^{3+} on the secondary microspheres, by diluting the supernatant containing the released Eu^{3+} ions and then performing one of the surface assays using the modified magnetic microspheres. Therefore, using the combination of the ion exchange microspheres and the mixed-ligand surface assays described above, it is possible to use these magnetic microspheres for detection of other ions than Eu^{3+}. Although the identity of these ions cannot be ascertained, their concentration can be estimated.

Discussion

The "physisorption" assays for Eu^{3+} yielded the greatest luminescence enhancement among the surface assays examined. The detriment of these assays was that high salinity must be maintained to prevent the aggregation of the magnetic microspheres. In solutions of low ionic strength, there was an ongoing aggregation of the magnetic microspheres that gradually decreased the luminescence yields and shortened the acquisition of the TRLF signal to a few minutes after mixing the reagents. Another problem was stripping the metal ions for the subsequent mass spectrometry analysis. Once the luminescent complex was formed at the surface at pH 4−7, re-extraction of Eu^{3+} into the aqueous phase using 0.01−1 M HNO_3 was slow and incomplete. Upon the increase in the acidity, these magnetic microspheres rapidly aggregated and the luminescent complex was trapped inside these aggregates. Since the addition of dispersing agents (e.g., detergents) removed the **2** into the aqueous phase, the covalently attached ligands provided a more robust platform.

For these surface-modified magnetic microspheres, clustering of neutral ligands using branched spacers (e.g., **8**), polymers (e.g., **11** and **12**), and dendrimers (e.g., **13**) *did not* show significant advantages over the simpler designs, either in terms of the extraction efficiency or luminescence enhancement. The best covalently attached ligand magnetic microsphere designs were at least 50−75% as efficient as the "physisorption" assays, but without the tendency to aggregate at low ionic strength. The greatest efficiency was shown by the magnetic microspheres in which the ligands were *directly* attached to the carboxyl and amino groups of the magnetic microspheres; the addition of spacers, whether hydrophilic or hydrophobic, had either weak effect or reduced the luminescence yield. The magnetic microsphere designs in which the polymers/dendrimers were first conjugated to the magnetic microspheres and then exhaustively labeled by the ligands yielded the largest luminescence enhancement with the longest luminescence lifetimes among the conjugates, suggesting efficient shielding of the luminescent complex from the aqueous solvent. All four classes of the ligands (**3**−**5**) performed equally well, although the conjugates of **4** and **5** demonstrated greater long-term chemical stability than the conjugates of **3**.

In all cases, the lifetime of the luminescence did not exceed 350−400 μs, often being as short as 200−250 μs, suggesting the presence of a luminescence quenching ligand. To estimate how many water ligands were involved, we replaced H_2O water by D_2O in extraction of Eu^{3+} by AMS-40-**5b** microspheres. The isotope replacement resulted in the increase of the lifetime from 216 to 295 μs, from which it was estimated, using the method of Nwe et al.,[13] that on average 1.5 water molecules are attached to the Eu^{3+} ion. Thus, the short luminescence lifetimes are the consequence of the relative permeability of the "hydrophobic" surface layers to water. Increasing the hydrophobicity of the coatings is problematic, as it leads to aggregation of the magnetic microspheres. Furthermore, the modified microspheres still have sulfonate groups at their surface increasing the polarity (and, subsequently, the water content) of this surface.

The performance of the ion-exchange assays (Figure 3) can be rationalized in terms of the single-type *f*-ion binding site on the surface of the microspheres. Let $[L]$ be the volume concentration of such binding sites and $[M_{1,2}]$ and $[m_{1,2}]$ be the free ion and total concentrations of the luminescent (index 1) and nonluminescent (index 2) *f*-ions, such as Eu^{3+} and Gd^{3+}, respectively, and $[LM_{1,2}]$ be the volume concentration of the bound ions. Then the concentrations can be determined from the following:

$$\begin{aligned} [M_{1,2}] &= m_{1,2}/(\beta_{1,2}[L] + 1) \\ [L] + [M_1] + [M_2] &= [L]_0 + [m_1] + [m_2] \end{aligned} \quad (1)$$

where $\beta_{1,2}$ are the corresponding binding constants. Solving these equations first for Eu^{3+}, using the experimental data on extraction of Eu^{3+}, allowed us to determine the maximum capacity $[L]_0$ for these ions and the binding constant β_1 and then use these parameters to estimate the optimum loading $[m_1]/[L]_0$ for the maximally linear response, i.e., the maximally linear initial range of the plot of $[M_1]$ vs $[m_2]$. For $\beta_1 \approx \beta_2$, numerical simulations indicate this optimum is at $[m_1]/[L]_0 \approx 0.65$. For the assay shown in Figure 3, this analysis gives the estimates of $[L]_0 \approx 4.6$ μM, $\beta_1[L]_0 \approx 60$, and $\beta_2[L]_0 \approx 1$. The first two estimates imply that the range of the reliable detection is 2−30 μM (0.5−6.0 $[L]_0$), in agreement with our results. As the

(13) Nwe, K.; Richard, J. P.; Morrow, J. R. *J. Chem. Soc., Dalton. Trans.* **2007**, 5171.

equilibria do not depend on the concentration of the microspheres (eq 1), this assay can be adapted to lower concentrations of Gd^{3+} by proportionally decreasing the concentration of the CMS-30 microspheres and Eu^{3+}. For each such concentration, the linearity window is the same, in the units of $[L]_0$.

Conclusions

We developed and demonstrated three approaches for sequestration, time-resolved luminescence detection, and subsequent mass spectrometry analysis of lanthanide ions using surface modified magnetic microspheres. One approach was using a DELFIA-type volume assays in which the ions are sequestered by aminocarboxylic ligands at the surface of the microspheres and the luminescence is developed in the volume bulk, inside the hydrophobic core of the micelles. The second approach is covering the surface of the microsphere with a monolayer of a hydrophobic, amphiphilic ligand, keeping the microsphere in the aqueous solution suspendable through the control of the salinity. The third approach is covalent linking of the ligands either directly to the microsphere surface or the "spacers" attached to this surface, and creating the hydrophobic environment conducive to the formation of the luminescent complex by covering this modified surface with nonionic detergent.

As these microspheres can be manipulated magnetically, these assays can be readily miniaturized and adapted for lab-on-the-chip devices. These approaches, therefore, provide a way for adapting the standard methods of liquid−liquid extraction and ion-exchange chromatography to microfluidic designs, by means of heterophase chemistry on suspendible microspheres. While the main thrust of this article was on the manipulation and detection of luminescent lanthanide ions, nonluminescent ions can be detected using the same schemes by taking advantage of ion-exchange properties of these sulfonated microspheres. We anticipate that these advances will speed up the adoption of microfluidics for general analytical and nuclear forensics applications.

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Supporting Information Available: Section 1S: Details of (A) TRLF measurements; (B) materials; (C) magnetic microsphere impregnation protocols; (D) synthetic methods; (E) conjugation protocols; (F) high-acidity adaptation of the assay; and (G) mass spectrometry measurements (PDF format). The material is available free of charge via the Internet at http://pubs.acs.org.

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