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Chemical Speciation of Trivalent Actinides and Lanthanides in Biological Fluids: The Dominant in Vitro Binding Form of Curium(III) and Europium(III) in Human Urine

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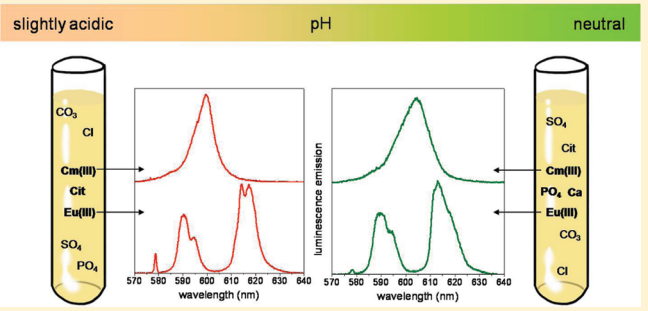
Supporting Information

ABSTRACT: Radionuclides represent a serious health risk to humans in the case of incorporation. To elucidate the potential of time-resolved laser-induced fluorescence spectroscopy (TRLFS) to determine the dominant radionuclide species in natural biofluids, we investigated the in vitro speciation of curium(III) in human urine samples. Because in speciation studies trivalent lanthanides are often used as analogues for trivalent actinides, we also probed the suitability of this theory by investigating the speciation of europium(III) in human urine. Comparison with reference spectra of both heavy metals in model urine and of their complexes with single organic and inorganic urine constituents then allowed for the determination of the dominant species. Furthermore, the chemical composition of all urine samples was analyzed, and the parameters affecting the speciation of the metals were determined. The pH was found to be the most important parameter because for both, the actinide and the lanthanide, two analogue species were identified in dependence on the pH. In samples with slightly acidic pH a curium(III) and europium(III) citrate complex dominates, respectively, whereas in samples with near-neutral pH a higher complex with phosphate and calcium as the main ligands and the additional participation of citrate and/or carbonate is formed in each case. Comparison with thermodynamic modeling yields some discrepancies, especially at higher pH, which is due to a lack of data for the complex formation of the higher species for both heavy metals. Nevertheless, TRLFS has proven to be a suitable method for the direct determination of the dominant heavy metal species in untreated natural human urine samples.



INTRODUCTION

Trivalent actinides (An(III)) such as curium(III) and americium(III) are mainly produced within the nuclear fuel cycle in nuclear power plants. Accidental release of such elements harbors the inherent danger of internal human contamination following acute or chronic exposure. This poses a serious health risk, because they have the potential to induce both radiological and chemical toxicity. Furthermore, whereas the majority of organic contaminants and poisons can be detoxified in vivo via metabolic degradation pathways, the human organism has only two ways to handle such toxic metal ions: immobilization or excretion.[1] No matter whether incorporation occurs via ingestion or inhalation or through the skin, the heavy metals are absorbed into and transported by the bloodstream prior to deposition in target organs. In the case of An(III), these are, in particular, the bones and liver with organ retentions of 30 and 50%, respectively.[2,3] In contrast, the excretion of those elements is <1% within the first 24 h and only 10% at the most within the first week.[2,4,5] Hence, elimination from the body is very slow, and the radionuclides exhibit long biological lifetimes of 20−50 years.[6] Due to the fact that all of the mentioned biological steps depend on kinetics and thermodynamic equilibria of the heavy metals with endogenous biological ligands, it is therefore highly important to know the speciation of the actinides in several body fluids and tissues to assess possible health risks. Because An(III) are excreted mainly via the kidneys,[4,6] it is their speciation in human urine that is of particular interest.

Because of the high specific radioactivity of these elements, the speciation of An(III) has prevalently been investigated with thermodynamic modeling in simulated body fluids such as bile, saliva, pancreatic fluid, and gastric juice.[7−9] Our own speciation calculations for curium(III) in human urine resulted in predominant citrate species up to pH 6.5 as well as in citrate and phosphate coordination at higher pH values.[10] The results and accuracy of such predictions rely significantly on the database and model used for calculation.[7] This means that if a naturally occurring species is unknown or the available data are incomplete, the calculation can be misleading and not coherent with the real speciation. Therefore, experimental studies with natural biological samples are essential.

Unfortunately, in case of An(III), hardly any in vivo speciation experiments have been performed due to the inherent ethical

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problems of such studies. Apart from the life-threatening Hanford Americium Accident,[11−13] just a handful of other accidental contamination cases are reported in the literature, particularly investigating the distribution patterns as well as the amounts of retained and excreted actinide.[5,14−17] Additionally, some animal studies with the same objective have been published.[18−20] In vitro speciation studies in natural biological samples are also quite rare for these elements. Whereas An(III) in human blood quickened some interest,[21,22] their speciation in other body fluids has yet to be clarified. Only Stradling et al. investigated the binding of curium(III) and americium(III) in urine with ultrafiltration and gel permeation chromatography and suggested citrate complexation for both radionuclides.[23] Nevertheless, the chemical form in which An(III) are excreted in the urine is not fully established,[4] and to the best of the authors' knowledge no other experimental study on their speciation in natural body fluids has been published.

As mentioned before, this lack of data is also due to the high requirements and risks inherent in experimental work with An(III). Therefore, trivalent lanthanides (Ln(III)) are often used as nonradioactive analogues. Both groups belong to the f-elements, which resemble each other due to similar ionic radii as well as identical oxidation states. Therefore, they are proposed to have similar properties and chemistry, which is termed the lanthanide actinide analogy. Furthermore, also the distribution patterns and biokinetics of these elements are similar. Europium(III), for instance, accumulates in bones and liver to 35 and 60%, respectively, and is excreted mainly via the kidneys.[24,25] Likewise, numerous distribution and excretion studies as well as speciation modeling in simulated body fluids were performed using Ln(III) as surrogates of An(III).[8,9,24,26]

In the present study, we therefore chose curium(III) as the representative An(III) as well as europium(III) as the corresponding Ln(III) analogue. Because both of these heavy metals exhibit unique luminescence properties, their speciation was investigated using time-resolved laser-induced fluorescence spectroscopy (TRLFS). This method is a highly suitable tool for this purpose, because it simultaneously provides information about the chemical state of an element directly out of its spectrum. From both single and time-dependent measurements, valuable parameters, which are sensitive to the chemical environment of the trivalent metal ions and vary in dependence on the ligand, are obtained. These are, for instance, emission wavelengths and luminescence lifetimes, which can be shifted and prolonged, respectively, upon complexation. The suitable application of the TRLFS method has been proven in previous studies on the complexation of curium(III) and europium(III) with various biological ligands in aqueous systems.[27−30] The aim of the present study was to elucidate the potential of TRLFS for direct heavy metal speciation analysis in untreated biological samples. Therefore, the dominant curium(III) and europium(III) species in natural human urine samples were determined for the first time. Furthermore, the suitability of the use of Ln(III) analogues was checked, and the parameters influencing the heavy metal speciation were identified.

EXPERIMENTAL PROCEDURES

Analysis and Characterization of Human Urine Samples. Human urine was collected from healthy volunteers over a period of 24 h or as spontaneous samples. The fresh samples were used without any further treatment and analyzed within 1 day after collection. Aliquots of each sample were stored at −20 °C. The inorganic composition was determined by mass spectrometry with inductive coupled plasma (ICP-MS) and ion chromatography (IC). With ICP-MS the content of metals and cations was measured using the ELAN 9000 system from Perkin-Elmer. The content of inorganic anions was determined with a HPLC system from Metrohm equipped with a Metrosep A Supp 4 column. As a sum parameter for the content of organic substances total organic carbon (TOC) was determined using a Multi N/C 2100 system from Analytik Jena AG. The pH was measured with glass electrodes from Mettler-Toledo (InLab 427 combination pH puncture electrode) or Schott (BlueLine 16 pH electrode). Additionally all samples were macroscopically characterized with regard to color, odor, and turbidity as well as analyzed with Medi-Test Combi 10 SGL control strips from Machery Nagel. Randomly, some of the urine samples were also studied microscopically with an Olympus BX 61 from Olympus at 400 times amplification in bright field and phase contrast.

Curium(III) and Europium(III) Speciation in Human Urine Samples. Two europium(III) stock solutions of 6.58×10^{-3} and 5×10^{-3} M were prepared by dissolving Eu_2O_3 (99.9%, trace metals basis, Aldrich) in 1 M $HClO_4$ and $EuCl_3 \cdot 6H_2O$ (Sigma, 99.999%) in carbonate-free, distilled water, respectively. In the case of curium(III) a 2.36×10^{-4} M stock solution of the long-lived ^{248}Cm isotope (half-life = 3.4×10^5 years) in 1 M $HClO_4$ was used. This radionuclide was supplied by the U.S. Department of Energy, Office of Basic Energy Sciences, via the transplutonium element production facilities at Oak Ridge National Laboratory. The isotope ratio of the solution was 97.3% ^{248}Cm, 2.6% ^{246}Cm, 0.04% ^{245}Cm, 0.02% ^{247}Cm, and 0.009% ^{244}Cm. From each urine sample aliquots of 3 mL were spiked in vitro with the actinide and lanthanide stock solution, respectively, so the final concentrations were 3×10^{-7} M curium(III) and 3×10^{-5} M europium(III). The pH was measured before as well as after the metal addition but was not readjusted. All urine samples were investigated with TRLFS within 1 h after labeling. Additionally, random urine samples spiked with europium(III) were centrifuged for 10 min at 2000 rpm [31], and the pellets were resuspended in carbonate-free, distilled water. Both the supernatant and the resuspension were then measured again with TRLFS and analyzed with ICP-MS as well as IC (as mentioned before).

Curium(III) and Europium(III) Speciation in Model Urine Solutions. Stock solutions of each 1 M NaCl (Merck, p.a.), KH_2PO_4 (Merck, p.a.), $CaCO_3$ (Merck, p.a.), $(NH_4)_2SO_4$ (Riedel-de Haen, p.a.), and $Mg(NO_3)_2 \cdot 6H_2O$ (Merck, p.a.) were prepared by dissolving the solids in carbonate-free, distilled water. With these a model urine solution containing the main inorganic but no organic ingredients of natural human urine was composed. The composition of this solution was based on our own ICP-MS and IC results of urine samples and literature data for standard urine compositions:[32−36] 90 mM sodium, 50 mM potassium, 3 mM calcium, 2 mM magnesium, 30 mM ammonium, 90 mM chloride, 50 mM phosphate, 30 mM sulfate, and 3 mM carbonate. Aliquots of 3 mL of model urine were spiked with the appropriate amount of metal stock solution, so the final concentrations were 3×10^{-7} M curium(III) and 3×10^{-5} M europium(III), respectively. The pH was measured before as well as after the metal addition. All samples were investigated with TRLFS within 1 h after spiking.

Curium(III) and Europium(III) Complexation with Model Ligands. Experimental details on the complexation with urea and amino acids are given elsewhere.[27,28] For citrate as the model ligand a stock solution of 1 M citric acid (Roth, water free, ≥99.5%, p.a., ACS) was prepared. To adjust the ionic strength a stock solution of 3 M $NaClO_4$ (Merck, analytical grade) was used as the background electrolyte. For all experiments the curium(III), europium(III), and citric acid concentrations were fixed to 3×10^{-7}, 3×10^{-5}, and 10^{-3} M, respectively. The pH was varied in a range relevant for human urine from 5 to 7,

Table 1. Chemical Composition of All 24-h Urine Samples (Contents in mM)

sample[a]	pH_0[b]	Na	K	Ca	Mg	Cl	PO_4	SO_4	CO_3	TOC
1 (f)	5.55	94	57	4.2	6.5	86	43	22	0.9	
2 (m)	5.73	188	108	4.3	3.9	166	57	23	0.2	
3 (f)	5.25	9	8	1.7	0.8	5	6	5	0.4	
4 (f)	6.75	116	79	0.8	1.3	111	13	10	5.9	
5 (f)	6.28	98	16	0.6	0.7	55	5	3	0.3	186
6 (m)	6.35	125	27	0.9	1.6	67	11	7	1.7	304
7 (f)	6.09	205	36	2.8	2.3	133	14	11	4.6	575
8 (m)	5.93	305	51	1.9	3.6	177	29	16	4.1	654
9 (f)	6.79	60	31	2.1	1.0	32	7	5	27	240
range	5.3−6.8	9−305	8−108	1−4	1−7	5−177	5−57	5−23	1−27	186−654
mean	6.1	133	46	2	2	92	21	11	5	392

[a] f, female; m, male. [b] pH of the original urine sample before metal addition.

and the ionic strength was kept constant at 0.1 M. TRLFS measurements of all samples were done within 1 h after preparation.

TRLFS Measurements. The time-resolved laser-induced fluorescence spectra were recorded using a pulsed flash lamp pumped Nd:YAG-OPO laser system (Powerlite Precision II 9020 laser equipped with a Green PANTHER EX OPO) from Continuum. Details on the experimental setup are given elsewhere.[29] The laser pulse energy was monitored using a photodiode, and the emission spectra were detected by an optical multichannel analyzer. This system consists of a monochromator (Oriel MS 257), a spectrograph with different gratings (300 and 1200 lines per mm, respectively), and an ICCD camera (Andor iStar); all components were purchased from the Lot-Oriel Group. A constant time window of 1 ms and an excitation wavelength of 395 nm were applied for all measurements. Curium(III) and europium(III) steady-state and time-dependent luminescence spectra were recorded in the 570−640 nm (grating = 1200 lines per mm with 0.2 nm resolution) and 450−750 nm (grating = 300 lines per mm) ranges, respectively. For time-resolved measurements a total of 35 spectra with delay steps of 10−100 μs were recorded per sample. In the case of curium(III) all experiments were run in a glovebox under nitrogen atmosphere, whereas europium(III) measurements were carried out under nitrogen as well as normal atmosphere. All experiments were performed at 24 ± 1 °C.

Data Analysis. TRLFS spectra were analyzed with Origin 7.5G[37] to obtain peak positions of luminescent species. Possible ligand field splitting was determined using the Peakfit Module with Lorentz and Gaussian fit functions for curium(III) and europium(III), respectively. To rule out fluctuations of the laser energy all TRLFS spectra were normalized to the same peak area of the curium(III) spectra. In the case of europium(III), normalization was applied to the intensity of the $^5D_0 \rightarrow {}^7F_1$ transition, because this is a magnetic dipole transition, and therefore it should be independent from the chemical environment of the lanthanide ion.[38,39] Furthermore, Origin was used to determine the lifetime of luminescent species according to the equation of exponential decay:

$$E(t) = \sum_i E_i \exp(-t/\tau_i) \quad (1)$$

In eq 1 E is the total luminescence intensity at the time t, E_i the luminescence intensity of species i at t, and τ_i the corresponding lifetime. With this lifetime the number of water molecules was calculated using the following equations given by Kimura et al.:[40]

$$n\mathrm{H_2O} \pm 0.5 = 0.65 \times \frac{1}{\tau} - 0.88 \text{ for curium(III)} \quad (2)$$

$$n\mathrm{H_2O} \pm 0.5 = 1.07 \times \frac{1}{\tau} - 0.62 \text{ for europium(III)} \quad (3)$$

RESULTS AND DISCUSSION

Analysis and Characterization of Natural Human Urine Samples. Fresh human urine samples were collected from nine healthy volunteers over a period of 24 h (samples 1−9) and from five healthy volunteers as spontaneous samples (samples 10−14). Of the 14 samples 10 originated from females (aged 21−51 years) and 4 from males (aged 27−31 years). Various tests including macroscopic characterization, control strips, and microscopic studies yielded standard values according to the literature[31,41] for each urine sample. Hence, no obvious pathological abnormalities that could alter the curium(III) and europium(III) speciation were observed. A summary of the test results is given in the Supporting Information.

The chemical composition of all urine samples is given in Tables 1 and 2. We concentrated on the main cations sodium, potassium, magnesium, and calcium as well as the anions chloride, phosphate, sulfate, and carbonate. The measured values represent the ordinary variety of element contents in human urine upon normal nutrition and are comparable to literature data.[32−36] All urine samples exhibit slightly acidic to near-neutral pH from 5.25 to 7.11 with a mean of 6.1 ± 0.2 for 24-h samples. For spontaneous samples the mean pH of 6.8 ± 0.1 appears to be higher. This is most likely due to the time of collection, which was at about 10 a.m. after breakfast. Therefore, it is possible that individual nutrition is responsible for the difference. By collecting the urine over a period of 24 h, such fluctuations are compensated. Nevertheless, this has not been investigated any further, and all urine samples were regarded as equal in this study.

Luminescence Spectra of Curium(III) and Europium(III) in Natural Human Urine Samples. Europium(III) spectra were recorded in all urine samples (samples 1−14), and fresh aliquots were used. For curium(III) spectra, stored 24-h urine samples were unfrozen at room temperature (samples 1−4), and for spectra in spontaneous samples fresh aliquots were used (samples 12−14). All urine samples were spiked with the heavy metal stock solution, and the pH was measured before and after the labeling. In each sample, the pH dropped to lower values due to the use of the acidified metal stock solution (see Table 3). Because, in general, this drop was quite low, the pH was not readjusted to the original value. Hence, all pH values mentioned in the following discussion refer to the pH of the urine samples after the metal addition. The luminescence spectra of europium(III) in natural human urine samples are depicted in Figure 1 and

Table 2. Chemical Composition of All Spontaneous Urine Samples (Contents in mM)

sample[a]	pH_0[b]	Na	K	Ca	Mg	Cl	PO_4	SO_4	CO_3	TOC
10(f)	6.77	20	10	4.3	0.7	22	2	2	23	107
11(f)	7.09	52	63	1.2	0.9	61	4	4	78	95
12(f)	6.37	46	9	1.3	0.5	40	1	3	28	77
13(f)	7.13	278	142	13.6	2.4	241	17	13	67	413
14(m)	6.75	280	109	12.2	1.5	243	14	14	134	593
range	6.4−7.1	20−280	9−142	1−14	1−3	22−243	1−17	2−14	23−134	77−593
mean	6.8	135	67	7	1	121	8	7	66	257

[a] f, female; m, male. [b] pH of the original urine sample before metal addition.

Table 3. Measured pH of the Urine Samples before and after Heavy Metal Addition

	Eu(III) in urine sample													
	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]	8[a]	9[a]	10[a]	11[a]	12[a]	13[a]	14[a]
pH_0	5.55	5.73	5.25	6.75	6.28	6.35	6.09	5.93	6.79	6.77	7.09	6.37	7.13	6.75
pH_{Eu}	5.45	5.64	4.80	6.53	5.70	6.09	5.89	5.78	6.56	6.34	6.64	5.49	6.98	6.30
ΔpH	0.10	0.09	0.47	0.22	0.58	0.26	0.20	0.15	0.23	0.43	0.45	0.88	0.15	0.45

	Cm(III) in urine sample						
	1[b]	2[b]	3[b]	4[b]	12[a]	13[a]	14[a]
pH_0	5.30	5.78	5.22	6.83	6.37	7.13	6.75
pH_{Cm}	5.27	5.71	5.21	6.79	5.77	6.96	6.70
ΔpH	0.03	0.07	0.01	0.04	0.60	0.17	0.05

[a] Fresh samples. [b] Stored samples (at −20 °C) unfrozen at room temperature.

those of curium(III) in Figure 2. The resulting spectroscopic parameters for europium(III) and curium(III) are summarized in Tables 4 and 5, respectively. On the basis of these parameters, two distinct groups of emission spectra were observed in dependence of the pH for both the actinide and the lanthanide.

Europium(III) steady-state spectra in urine samples with pH ≤5.7 after metal addition (samples 1−3, 5, and 12) are strikingly similar to one small emission peak at 579 nm and two broad luminescence bands at 592 and 617 nm, which are both split (see Table 4). The luminescence intensity ratio of the two broad peaks (I_1:I_2) is 1:2.2 ± 0.4. In the investigated wavelength range of 570−640 nm, the spectrum of the Eu^{3+} aqua ion exhibits only the two luminescence bands at 592 and 617 nm[27,28,42,43] as well as an intensity ratio of 1:0.5. Hence, the steady-state spectrum of europium(III) in human urine samples differs significantly from that of the aqua ion with regard to the occurrence of the first peak and the splitting pattern of the second and third peaks as well as the intensity ratio. Analysis of the time-resolved measurements gave monoexponential luminescence decay in all urine samples with pH ≤5.7. The emission lifetimes range from 128 to 176 μs and can be averaged to 153 ± 19 μs. Applying the equation of Kimura et al.,[40] these values correspond to five to seven water molecules in the first hydration sphere of the metal ion. Compared to the Eu^{3+} aqua ion, which has an emission lifetime of 110 ± 10 μs corresponding to nine water molecules,[27,28,42,43] this means the replacement of two to four of these molecules by urinary ligands. Hence, all spectroscopic parameters indicate the formation of the same *Eu(III) urine complex 1* in all human urine samples with pH ≤5.7.

In contrast, all europium(III) spectra in urine samples with pH ≥5.8 after metal addition (samples 4, 6−11, 13, and 14) are significantly different from that of the *Eu(III) urine complex 1*, but quite identical among themselves. These spectra are characterized by another splitting pattern of the second and third emission peaks at 592 and 617 nm, respectively (see Figure 1), and a lower intensity ratio with 1:1.6 ± 0.3. Furthermore, the first luminescence peak is much less pronounced than in the spectrum of the *Eu(III) urine complex 1*. This indicates the formation of another *Eu(III) urine complex 2* in human urine samples with pH ≥5.8. Time-resolved luminescence curves showed monoexponential decay for all of those samples and resulted in emission lifetimes varying widely from 350 to 1190 μs, with an average of 582 ± 285 μs. These lifetimes equal a number of two water molecules at the most in the first coordination shell of the metal ion. Hence, nearly all water molecules of the Eu^{3+} aqua ion have been replaced by urinary ligands in the *Eu(III) urine complex 2*. On the basis of the spectroscopic parameters, both urinary europium(III) complex species are very well distinguishable.

The steady-state luminescence spectra of curium(III) in human urine samples with pH ≤5.8 after metal addition (samples 1−3 and 12) are nearly identical and exhibit an emission maximum at 600 nm. Because the emission spectrum of the Cm^{3+} aqua ion peaks at 593.5 nm,[27,29,44−46] this means a red shift of the curium(III) luminescence wavelength in human urine with pH ≤5.8. Time-resolved measurements exclusively yielded monoexponential decay curves in urine samples with pH ≤5.8. The lifetimes range between 119 and 125 μs and can be averaged to 122 ± 3 μs. According to Kimura et al.,[40] these values correspond to four to five water molecules in the first coordination

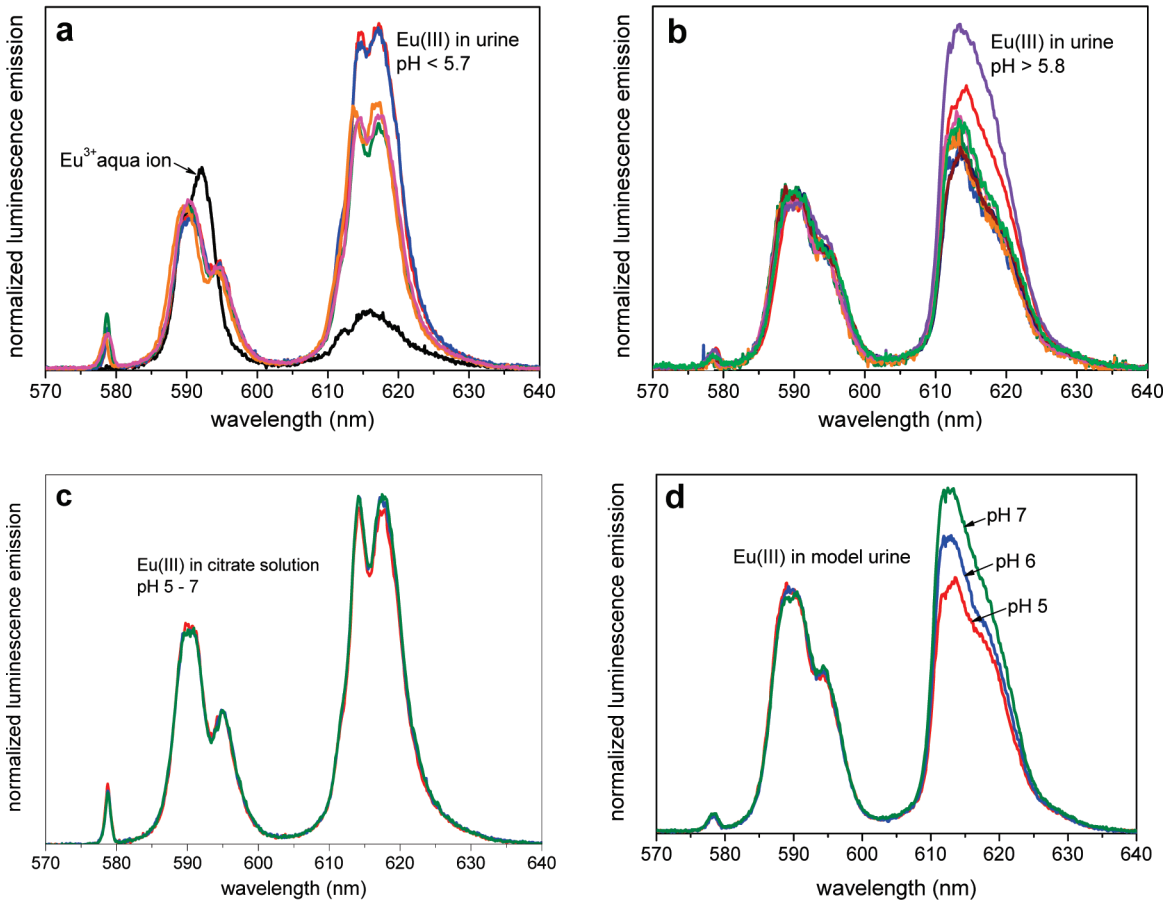

Figure 1. Luminescence spectra of 3×10^{-5} M europium(III) in human urine samples with pH ≤5.7 (a) and pH ≥5.8 (b) as well as in 10^{-3} M aqueous citrate solution at pH 5−7 (c) and model urine (d).

sphere of the metal ion. Because the Cm^{3+} aqua ion has a luminescence lifetime of 68 ± 1 μs,[27,29,44−46] equaling nine water molecules, four to five of them have been replaced upon complexation. Therefore, the spectroscopic parameters indicate the formation of the same *Cm(III) urine complex 1* in all urine samples with pH ≤5.8.

In contrast, all curium(III) emission spectra recorded in urine samples with pH ≥6.7 after metal addition (samples 4, 13, and 14) differ significantly from that of the *Cm(III) urine complex 1*, but are very similar to one another. These curium(III) spectra exhibit a broad maximum at 605 nm, which means an even larger red shift of the emission wavelength compared to the Cm^{3+} aqua ion than in the *Cm(III) urine complex 1*. Furthermore, all of these spectra have smaller emission intensities and are much noisier than those in human urine with lower pH. Time-resolved measurements resulted in biexponential decay for urine samples 4 and 14, whereas that in sample 13 is monoexponential. The shorter lifetimes in urine samples 4 and 14 were determined to be 71 and 143 μs, respectively, and the longer lifetimes are 466 and 470 μs, respectively. Therefore, the shorter values correspond to the Cm^{3+} aqua ion in sample 4 and the *Cm(III) urine complex 1* in sample 14, whereas the longer lifetime is assigned to the new *Cm(III) urine complex 2*. The emission lifetime of curium(III) in urine sample 13 was determined to be 538 μs. The monoexponential decay curve indicates that only the *Cm(III) urine complex 2* is formed in this sample. The average lifetime of 491 ± 40 μs for this complex corresponds to one water molecule, at most, left in the first hydration shell of the Cm^{3+} ion. Hence, nearly all water molecules have been replaced by urinary ligands. Just like in case of the lanthanide, both urinary curium(III) complex species are very well distinguishable from each other by their spectroscopic parameters.

Luminescence Spectra of Curium(III) and Europium(III) Complexes with Model Ligands. Because the complexation of the actinide and the lanthanide with the main inorganic anions of human urine is well studied[47,48] but data on the complexation with organic model ligands are mainly missing, we also aimed at addressing this lack of knowledge. It is well-known that in human urine the main organic component is urea with a concentration of 0.1−0.5 M.[35,49] The second main group comprises organic acids, of which citric acid is contained in amounts of $(0.5-2.5) \times 10^{-3}$ M.[35,36,50] Furthermore, amino acids are ubiquitous in all body fluids and occur in human urine at 10^{-4}−10^{-3} M.[35,51] Therefore, the complexation of curium(III) and europium(III) with these model ligands has been studied in aqueous solution, and reference spectra at I = 0.1 M as well as urine-relevant concentrations and pH were recorded.

The complexation with the organic matrix substance urea was studied at urine-relevant concentrations and pH.[27] For both heavy metals, the formation of a 1:1 complex was observed (emission spectra and spectroscopic parameters are given in the Supporting Information). The curium(III) complex exhibits a luminescence wavelength at 599 nm and an emission lifetime of 80−90 μs, which corresponds to seven water molecules in the

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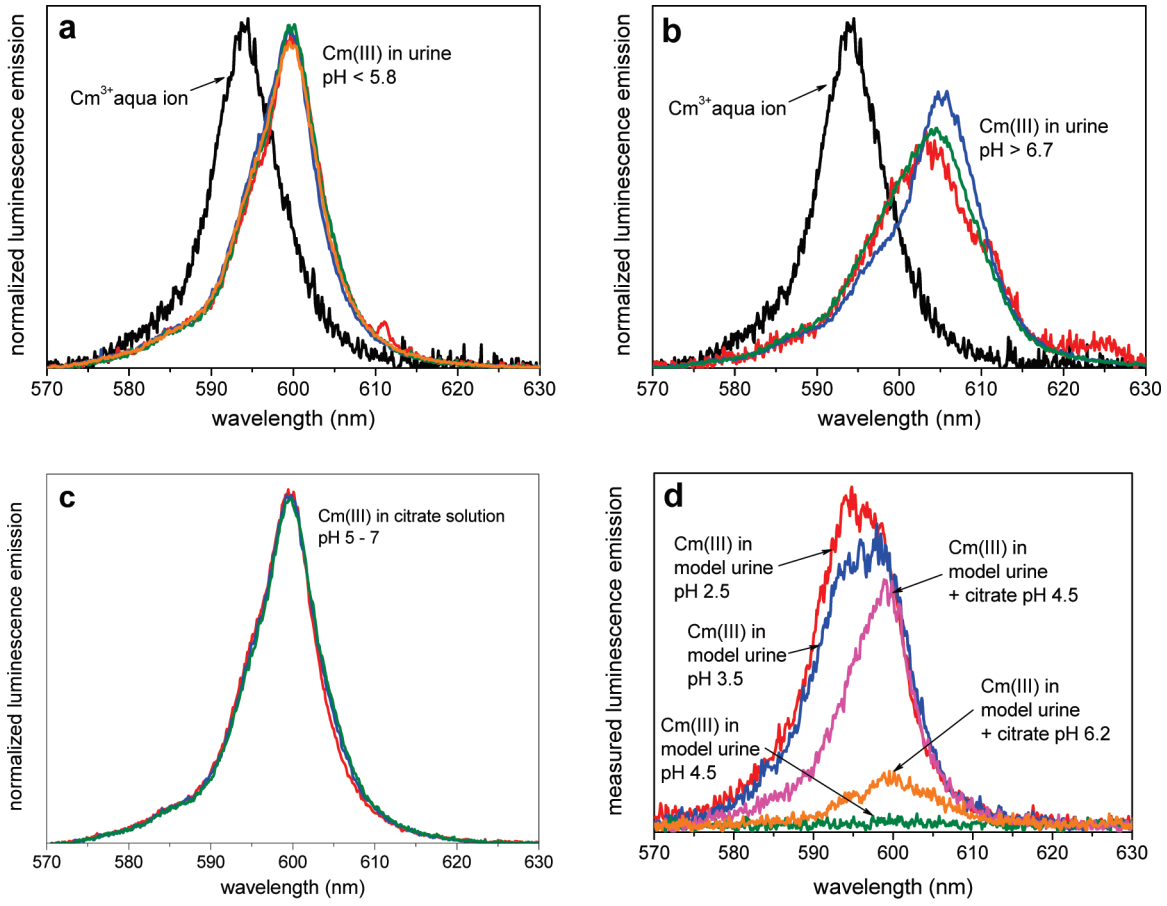

Figure 2. Luminescence spectra of 3×10^{-7} M curium(III) in human urine samples with pH ≤5.8 (a) and pH ≥6.7 (b) as well as in 10^{-3} M aqueous citrate solution at pH 5−7 (c) and model urine (d).

Table 4. Spectroscopic Parameters of the Europium(III) Luminescence in Human Urine and Model Solutions

	emission wavelengths (nm)					
Eu(III) solution	$^5D_0 \rightarrow {}^7F_0$	$^5D_0 \rightarrow {}^7F_1$	$^5D_0 \rightarrow {}^7F_2$	I_1:I_2[a]	lifetime (μs)	ref
Eu(III) in water (Eu^{3+} aqua ion)		592	616	1:0.5	112 ± 3	this work
		593	617		110 ± 10	42, 43
Eu(III) in human urine, pH ≤5.7[b]	579	590/595	614/617	1:2.2	153 ± 19	this work
Eu(III) + citrate, pH 5−7	579	590/594	614/617	1:2.0	260 ± 7	this work
Eu(III) + citrate, pH 3.6					180 ± 6	53
Eu(III) in human urine, pH ≥5.8[c]	578	591/594[d]	613/617[d]	1:1.6	582 ± 285	this work
Eu(III) in model urine, pH 5−7	578	592/594[d]	613/617[d]	1:1.4	271 ± 64[e]	this work
Eu(III) in model urine + citrate pH 5−7	578	592/595[d]	614/617[d]	1:1.4	353 ± 18[e]	this work
Eu(III) + phosphate, pH 6−7	578	592/594[d]	614/617[d]	1:1.1	278 ± 8	this work
Eu(III) + phosphate, carbonate, citrate, and calcium, pH 6	578	591/594[d]	614/617[d]	1:1.3	563 ± 14	this work

[a] Intensity ratio of the transitions into the $^7F_1$ and $^7F_2$ ground states (intensity of the $^5D_0 \rightarrow {}^7F_1$ transition peak is fixed as 1). [b] Samples 1−3, 5, and 12. [c] Samples 4, 6−11, 13, and 14. [d] Shoulder. [e] pH-dependent (slight prolonging with increasing pH).

first coordination sphere of the metal ion. The luminescence spectrum of the analogue europium(III) complex is characterized by two broad bands at 592 and 612 nm with a unique splitting pattern and an intensity ratio of 1:1.5 ± 0.1. The lifetime of this complex is 145 ± 2 μs and equals seven water molecules as in the case of the actinide. Hence, the spectra of the curium(III) and europium(III) urea complex differs significantly from those of the heavy metals in natural human urine. Stability constants and a detailed discussion are given elsewhere.[27]

Investigation of the complexation of europium(III) with alanine, phenylalanine, and threonine at urine-relevant pH and ligand concentrations demonstrated the formation of 1:1 complexes with the zwitterionic form of each amino acid (emission spectra and spectroscopic parameters are given in the Supporting Information).[28] The steady-state luminescence spectra of the three complexes are characterized by splitting patterns significantly different from those of the urea complexes but also different from those in human urine. Analysis of the time-resolved

Table 5. Spectroscopic Parameters of the Curium(III) Luminescence in Human Urine and Model Solutions

Cm(III) solution	emission wavelength (nm) $^6D_{7/2} \rightarrow {}^8S_{7/2}$	lifetime (μs)	ref
Cm(III) in water (Cm^{3+} aqua ion)	594	67 ± 1	this work
	594	68 ± 1	29, 44−46
Cm(III) in human urine, pH ≤ 5.8[a]	600	122 ± 3	this work
Cm(III) + citrate, pH 5−7	600	134 ± 6	this work
Cm(III) in human urine, pH ≥ 6.7[b]	605	491 ± 40[c]	this work
Cm(III) in model urine, pH 2−4	599	76 ± 3	this work
Cm(III) + phosphate, pH 1.5	600	71 ± 3	56
Cm(III) in model urine + citrate, pH 4−6	600	115 ± 1	this work
Cm(III) + phosphate, pH 5−6 (colloidal)	603	220 ± 20	56

[a] Samples 1, 2, 3, and 12. [b] Samples 4, 13, and 14. [c] Samples 4 and 14 show biexponential luminescence decay; the longer lifetime was used to calculate the average lifetime.

measurements resulted in monoexponential luminescence decay, and lifetimes were determined to be 127 ± 3 μs for each complex, which equals eight water molecules in the first hydration shell of the Eu^{3+} ion. Therefore, also the spectra of europium(III) with amino acids do not match those recorded in natural human urine. A detailed discussion and the stability constants of the several complexes are given elsewhere.[28]

Due to its ubiquitous occurrence in body fluids, citrate was chosen as the third model ligand. At urine-relevant pH, complexation with this ligand has been observed for both metals. As can be seen in Figures 1c and 2c, for each element one identical luminescence spectrum was recorded in the range of pH 5−7. The emission wavelength of the curium(III) complex is 600 nm, and the monoexponential luminescence decay yielded a lifetime of 134 ± 6 μs. This corresponds to six water molecules left in the first hydration shell of the Cm^{3+} ion and the replacement of three molecules by the ligand. The emission spectrum of the europium(III) complex exhibits a sharp peak at 579 nm and two broad bands at 592 and 617 nm, which are split. The luminescence lifetime was determined to be 260 ± 7 μs, equaling five water molecules left and four that have been replaced by the ligand. All spectroscopic parameters equal those of the heavy metals in natural human urine with slightly acidic pH and are given in Tables 4 and 5 for europium(III) and curium(III), respectively.

The complexation studies with model ligands showed that at urine-relevant pH 5−7, both curium(III) and europium(III) form only one complex with each of the respective ligands. The reported stability constants of the complexes indicate that urea is the weakest ligand followed by the zwitterionic amino acids, whereas citrate is a strong ligand.[27,28,52] From that point of view, urea and amino acids should not be relevant for the heavy metal binding in human urine, but citrate can be expected to play a role in the speciation of both elements in human urine. This is confirmed by our results because the emission spectra of the respective urea and amino acid complexes revealed no congruence to those of both heavy metals in natural urine samples. In contrast, the curium(III) and europium(III) citrate spectra are very similar to those of the actinide and lanthanide recorded in human urine with a slightly acidic pH.

Luminescence Spectra of Curium(III) and Europium(III) in Model Urine Solutions. Because none of the complexes with model ligands gave emission spectra similar to those of the heavy metals in human urine samples with near-neutral pH, a model urine solution was synthesized to check whether inorganic complexes would fit the urinary spectra. This model urine contained the main inorganic but no organic components of human urine, and the composition was based on our own ICP-MS and IC results of urine samples 1−9. Europium(III) and curium(III) luminescence spectra in inorganic model urine at different pH values are depicted in Figures 1d and 2d, respectively. The spectroscopic parameters are summarized in Table 4 for europium(III) and in Table 5 for curium(III).

The luminescence of curium(III) is enhanced in the model solution at pH 2.5 compared to that of the Cm^{3+} aqua ion. Increasing the pH resulted in a slight decrease of the emission intensity to pH 3.5 and then in a quick and drastic drop to around pH 4. Therefore, at pH 4.5, no significant emission spectrum was measurable anymore. Upon addition of 10^{-3} M citric acid at pH 4.5, the curium(III) luminescence reappeared, but further increase of the pH resulted once more in a decrease of the emission intensity. The steady-state spectrum of curium(III) in inorganic model urine solution is characterized by a broad emission maximum around 596 nm, which is split into two single peaks at 594 and 599 nm. Whereas the first peak is identical with the luminescence maximum of the Cm^{3+} aqua ion, the second is assigned to the complex formed in the model solution. After the addition of citric acid, the emission spectrum is sharpened and the maximum is slightly shifted to 600 nm. In time-resolved measurements, monoexponential decay curves were observed for all curium(III) spectra in model urine. In inorganic model solution, the lifetime is 76 ± 3 μs, and after the addition of citric acid it is prolonged to 115 ± 1 μs.

In the case of europium(III), luminescence spectra are strikingly similar at pH 5−7, with one small peak at 578 nm and two broad emission bands at 592 and 617 nm, which are both split into each two single peaks (see Table 4). The intensity ratio is 1:1.4 ± 0.2 for all samples with a slight enhancement upon increased pH. Analysis of the time-resolved measurements resulted in monoexponential decay curves and successively prolonging lifetimes with increasing pH from 180 μs at pH 5 to 306 μs at pH 7. These values correspond to three to five water molecules in the first coordination sphere of the Eu^{3+} ion and the replacement of up to six molecules by ligands of the model urine solution. Addition of citric acid to europium(III) in model urine solution, on the one hand, resulted in no significant changes of the steady-state spectra at any pH. On the other hand, a prolonging of the emission lifetime upon addition of citric acid was observed. Hence, at pH 5, the lifetime is 335 μs, and at pH 7, it is 370 μs. These values equal a number of two to three water molecules remaining in the first hydration shell of the metal ion.

This means that upon addition of citric acid to the model urine solution more water molecules are displaced in the europium(III) complex.

Determination of the Dominant Curium(III) and Europium(III) Species in Human Urine. The dominant urinary species of both the actinide and the lanthanide were determined via comparison of the luminescence spectra measured in natural human urine samples with those recorded in aqueous solutions of various model ligands and model urine.

For europium(III), this revealed striking similarities between the luminescence spectra in human urine samples with pH ≤5.7 and those in aqueous citrate solution. It is obvious from Figure 1 that all of these steady-state spectra exhibit identical splitting patterns of the emission peaks and very similar luminescence intensity ratios (see Table 4). Unfortunately, the results of the time-dependent measurements are not so consistent because the emission lifetime in citrate solution is somewhat longer than that in urine samples with pH ≤5.7. Nevertheless, when compared to the lifetime of europium(III) in citrate solution at pH 3.6, which was determined to be 180 ± 6 μs by Mathur et al.,[53] the value obtained for europium(III) in urine samples with pH ≤5.7 is in good agreement. After centrifugation of a representative urine sample with pH ≤5.7 (sample 3), the metal content of the pellet was negligible (8×10^{-7} M) and all europium(III) was detected in the supernatant (3×10^{-5} M). Furthermore, the emission spectrum measured in the supernatant is identical to that measured in the original sample. These findings and all spectroscopic parameters demonstrate that the *Eu(III) urine complex 1* formed in human urine samples with pH ≤5.7 is a water-soluble citrate complex.

The steady-state europium(III) spectra in human urine samples with pH ≥5.8 closely resemble those in inorganic model urine solution. As can be seen in Figure 1 and Table 4, the splitting pattern and the intensity ratio are nearly identical for all of these samples. In contrast, comparing the luminescence lifetime yielded some discrepancies. Because the values obtained in human urine samples with near-neutral pH vary over a wide range, they occasionally are significantly longer than those measured in inorganic model urine solution. Upon centrifugation of a representative urine sample with pH ≥5.8 (sample 9), all europium(III) was detected in the precipitated pellet (3×10^{-5} M) and only traces were found in the supernatant (3×10^{-7} M). Furthermore, the emission spectrum of the resuspended pellet equaled that measured in the original sample. This indicates the formation of an insoluble complex, which is present in colloidal form in human urine samples with pH ≥5.8. Because the citrate complex was shown to be water-soluble, this indicates the binding of europium(III) by inorganic ligands at near-neutral pH. Therefore, luminescence spectra of europium(III) with each of the inorganic anions were recorded at pH 6−7 and concentrations equaling those of the model urine (steady-state spectra given in the Supporting Information). Carbonate, sulfate, and chloride yielded emission spectra significantly different from that of *Eu(III) urine complex 2*. In contrast, the luminescence spectrum with phosphate as the ligand is almost identical to that of europium(III) in model urine and to that of *Eu(III) urine complex 2* (see Supporting Information). Unfortunately, the time-resolved measurements reveal differences because, in phosphate solution, two different europium(III) complexes exist. The species with the longer lifetime of 278 ± 8 μs, on the one hand, equals the europium(III) complex formed in inorganic model urine. On the other hand, it does not resemble the species formed in natural urine samples with pH ≥5.8, which has a significantly longer lifetime. Nevertheless, these findings clearly demonstrate that phosphate is the main complexing ligand of europium(III) in both inorganic model urine and natural human urine with pH ≥5.8. Further support for this is given by the poor water solubility reported for europium(III) phosphate ($\log K^0_{sp} = -25 \pm 1$),[54,55] which is in agreement with the insolubility and colloidal nature of *Eu(III) urine complex 2*. However, the long lifetimes of europium(III) in human urine with pH ≥5.8, which correlate to the exclusion of nearly all water molecules in the first hydration shell of the Eu^{3+} ion, indicate the binding of at least one more ligand in *Eu(III) urine complex 2* and the formation of a ternary or higher complex species.

To verify the formation of this higher complex luminescence spectra of europium(III) with phosphate and carbonate (solution 1), phosphate and citrate (solution 2) as well as phosphate, carbonate, and citrate were recorded (solution 3). All steady-state spectra are almost identical to those of europium(III) in phosphate solution, model urine, and natural human urine with pH ≥5.8 (see Supporting Information). Analysis of the time-resolved spectra resulted in very similar emission lifetimes in all three solutions, which are, moreover, close to the longer lifetime in pure phosphate solution. If solution 3 was prepared with one of the additional ligands as its calcium salt, the luminescence spectrum shows significant differences. On the one hand, the emission intensity of the third peak is enhanced. This leads to an increase of the intensity ratio to 1:1.3, which is close to the value of *Eu(III) urine complex 2*. On the other hand, the luminescence lifetime is significantly prolonged to 563 ± 14 μs and in agreement with the average lifetime of europium(III) in human urine with pH ≥5.8. Hence, these measurements clearly show that also calcium is participating in the formation of *Eu(III) urine complex 2*. Whether carbonate and/or citrate is also involved is not certain from these measurements and needs further investigation.

Curium(III) luminescence spectra in urine samples with pH ≤5.8 are closely matched by those in aqueous citrate solution. As can be seen in Figure 2 as well as Table 5, all of these spectra exhibit identical emission maxima, and the luminescence lifetimes in these solutions are also very similar. All findings and spectroscopic parameters, therefore, clearly demonstrate that the *Cm(III) urine complex 1* formed in human urine samples with pH ≤5.8 is a citrate complex, just like in case of the lanthanide. Comparison of the steady-state spectra of curium(III) in urine samples with pH ≥6.7 resulted in no analogy because none of the spectroscopic parameters were matched by the reference spectra in inorganic model urine or model urine with citrate addition. However, with regard to the lanthanide actinide analogy, curium(III) should form a similar ternary or higher complex species such as europium(III) in human urine samples with near-neutral pH. Unfortunately, there is a lack of complex and solubility data of curium(III) phosphate compounds. Recently, this has been addressed by Moll et al., who report on the curium(III) complexation by phosphate using TRLFS.[56] The authors provide luminescence spectra and the corresponding spectroscopic parameters for the dihydrogen- and hydrogenphosphate complexes and demonstrate the existence of colloid curium(III) phosphate species at millimolar concentrations and pH ≥5.[56] The published emission wavelength and lifetime of the $CmH_2PO_4^{2+}$ complex, which forms at pH 1.5, are very similar to our own data of curium(III) in inorganic model urine solution at pH 2−4 (see Table 5). This indicates the

formation of phosphate complexes in inorganic model urine. Thermodynamic modeling of the Cm(III) phosphate system indicates the formation of the insoluble $CmPO_4$ species at around pH 5,[56] which explains the TRLFS results in inorganic model urine. Furthermore, the luminescence wavelength and lifetime of the curium(III) phosphate colloids reported by Moll et al.[56] are closer to those of curium(III) in human urine samples with pH ≥6.7 than any other reference data (see Table 5). On the one hand, these findings demonstrate that phosphate is involved in curium(III) complexation in both model urine and natural human urine with pH ≥6.7. On the other hand, the remaining discrepancies strongly suggest the binding of an additional ligand in *Cm(III) urine complex 2* and the formation of a ternary or higher complex. This hypothesis is supported by the extremely long lifetime of this complex species, which indicates the replacement of nearly all water molecules in the first hydration shell of the Cm^{3+} ion and points to the complexation with more than one type of ligand. Last but not least, this would also be in analogy with the lanthanide.

To summarize, the curium(III) citrate complex is characterized by a smaller emission wavelength and a narrow peak shape compared to the higher complex species. The spectrum of the europium(III) citrate species is characterized by the significant appearance of the first peak at 579 nm, the distinct splitting patterns of the second and third emission bands, and the higher intensity ratio compared to the higher complex. Furthermore, the citrate species of both heavy metals exhibit shorter lifetimes than do the respective higher complexes and indicate the exclusion of five water molecules at most from the first hydration shell of the metal ions upon complexation. In contrast, the very long lifetimes of the higher complex species indicate the replacement of nearly all water molecules by urinary ligands for both the actinide and the lanthanide. Those higher complexes presumably consist of inorganic as well as organic ligands. Of the various potential urinary ligands, phosphate has clearly been demonstrated to be involved in the case of both elements. Furthermore, europium(III) experiments give also strong indications for the involvement of calcium. Whether, additionally, carbonate and/or citrate are complexed in these species, could not be established without doubt in this study.

CONCLUSIONS

For the first time, the in vitro urinary speciation of curium(III) and europium(III) as representatives of An(III) and Ln(III), respectively, was investigated using TRLFS. Emission spectra and lifetimes of both elements in various samples were recorded and demonstrated the suitability of this method for direct fluorescence spectroscopic determination of the dominant heavy metal species in untreated natural human urine samples.

We demonstrated that both elements form water-soluble citrate complexes as well as ternary or higher species in human urine dependent on the pH. Therefore, our results, on the one hand, spectroscopically confirm the initial suggestion by Stradling et al. that curium(III) is bound to naturally occurring citrate in human urine.[23] On the other hand, we also demonstrated the occurrence of another complex species in some of the natural samples. Furthermore, the present study points out the importance of the pH of the sample, because curium(III) and europium(III) citrate complexes were identified in urine samples with slightly acidic pH, whereas the respective higher complex species were detected in urine samples with near-neutral pH. This is in agreement with thermodynamic modeling of curium(III) and americium(III) speciation in body fluids, which result in the dominance of complexes with organic ligands at slightly acidic pH and that of inorganic species at near-neutral pH.[8,9] Compared to our own speciation calculations of curium(III) and europium(III) in human urine,[10] we discovered good correlation with the experimental data in the slightly acidic region, where the modeling predicts nearly 100% citrate complexation for both the actinide and the lanthanide. In the range of near-neutral pH, the speciation calculation predicts the simultaneous occurrence of two complexes for each heavy metal, namely, 56% citrate coordination and about 42% phosphate complexation. Hence, this reveals some discrepancies between model and experimental results in this pH range. This can easily be explained by the incomplete database of the curium(III) and europium(III) speciation models due to a lack of formation and solubility constants of the respective higher complex species present in natural urine samples with near-neutral pH.

With regard to the lanthanide actinide analogy, the present study shows that curium(III) and europium(III) form analogue complexes in each solution investigated. Some discrepancies, namely, with experiments in model urine solution, can be attributed to the different heavy metal concentrations and low solubility of the formed species.

To summarize, both experimental and simulated data indicate that in biological fluids at acidic pH curium(III) and europium(III) are bound by organic ligands and preferably by citrate. In contrast, the speciation of both heavy metals in biofluids at neutral pH is dominated by inorganic complexes. This, in turn, demonstrates that it is not sufficient to take only the inorganic composition of a respective biofluid into account as done by some previous studies[57] but that organic substances play a crucial role and have to be considered, too.

ASSOCIATED CONTENT

Supporting Information. Macroscopic characterization of human urine samples and results of the control strips, spectroscopic parameters and emission spectra of Cm(III) and Eu(III) in model ligand solutions, luminescence decay curves of Cm(III) and Eu(III) in human urine samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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