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Speciation of the trivalent f-elements Eu(III) and Cm(III) in digestive media

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Abstract

In case radioactive materials are released into the environment, their incorporation into our digestive system would be a significant concern. Trivalent *f*-elements, i.e., trivalent actinides and lanthanides, could potentially represent a serious health risk due to their chemo- and radiotoxicity, nevertheless the biochemical behavior of these elements are mostly unknown even to date. This study, therefore, focuses on the chemical speciation of trivalent *f*-elements in the human gastrointestinal tract. To simulate the digestive system artificial digestive juices (saliva, gastric juice, pancreatic juice and bile fluid) were prepared. The chemical speciation of lanthanides (as Eu(III)) and actinides (as Cm(III)) was determined experimentally by time-resolved laser-induced fluorescence spectroscopy (TRLFS) and the results were compared with thermodynamic modelling. The results indicate a dominant inorganic species with phosphate/carbonate in the mouth, while the aquo ion is predominantly formed with a minor contribution of the enzyme pepsin in the stomach. In the intestinal tract the most significant species are with the protein mucin. We demonstrated the first experimental results on the chemical speciation of trivalent *f*-elements in the digestive media by TRLFS. The results highlight a significant gap in chemical speciation between experiments and thermodynamic modelling due to the limited availability of thermodynamic stability constants particularly for organic species. Chemical speciation strongly influences the *in vivo* behavior of metal ions. Therefore, the results of this speciation study will help to enhance the assessment of health risks and to improve decorporation strategies after ingestion of these (radio-) toxic heavy metal ions.

Keywords

trivalent *f*-elements, actinides and lanthanides, time-resolved laser-induced fluorescence spectroscopy (TRLFS), metal ion speciation, simulated digestive system, Unified Bioaccessibility Method (UBM)

1. Introduction

Actinides (An) are a series of radioactive heavy elements, some of which are the main fuel components for nuclear power generation. Lanthanides (Ln) are the non-radioactive chemical analogs to An with a wide range of technological applications in, for instance, catalytic, magnetic, electronic or medical fields. Due to their chemo- and radiotoxicity these elements could represent a serious health risk [1-4]. As a matter of fact, several incidents that resulted in the human exposure to these heavy metals have been reported, for instance, accidental release from nuclear power plants, waste repositories or from mining activities. [5, 6]. In case the human body is exposed to radioactivity or toxic substances, the material could be incorporated and further transported through the digestive system from the mouth to the stomach and intestine. After the intestinal absorption the nutrients will be further transported to the blood circulation and eventually reach target organs (e.g., bones, kidney, liver), where they can be stored or directly eliminated through urine and faeces [7, 8].

The actinides and lanthanides (namely “*f*-elements”) have no essential role in the human body. However, the excretion of these heavy metal ions from the human body is very slow (<1 % within the first 24 h and 10 % within the first week) [2, 9, 10]. Assuming their long biological lifetimes of 20-50 years [11], they could interact with endogenous biological ligands to form complexes that could be absorbed and transported to the bloodstream [12] once they are introduced into the human body. In order to assess their toxicity and potential health risks, as well as to develop effective decontamination measures [5, 7, 11, 13-15], it is essential to understand the speciation of these elements in human body fluids, as the chemical speciation could significantly affect their stability and mobility *in vivo*, further influencing their bio-availability and metabolism. The speciation of trivalent *f*-elements under *in vivo* conditions is, however, mostly unknown even to date. This motivates us to perform the present study to investigate the chemical speciation of Cm(III) and Eu(III) in human digestive juices, one of the essential body fluids of humans.

The precedent studies reporting the speciation of *f*-elements in human body fluids have been limited for urine and blood compartments (e.g., transferrin) *in vitro* or in animals [9, 14-21]. Furthermore there are several studies on the speciation in some specific organs such as liver and bone [15, 22, 23]. There are a very limited number of studies on the speciation of An in the human gastrointestinal tract. For instance, the speciation of hexavalent uranium (U(VI))

and Cm(III)/Eu(III) was studied in saliva [21, 24]. The *in vitro* absorption of tetravalent neptunium (Np(IV)) was reported in the small intestine, suggesting that the complexation of Np(IV) with biological ligands would have a strong influence on the absorption behavior of this radionuclide in the digestive system [25].

Chemical speciation can be also simulated by thermodynamic models. However, the reliability of such thermodynamic calculations depends largely on the reliability and availability of the data reported at the time of study. For instance, Webb et al. reported the speciation modelling for Ln(III) (Gd and Eu) and An(III) (Am and Cm) in the simulated body fluids from mouth, stomach and small intestine [26, 27]. Given the chemical similarity between Ln(III) and An(III), the speciation of these elements are expected to be similar. However, the study by Webb et al. reported different speciations for Ln(III) and An(III). The source of the stability constants used in their thermodynamic modelling was not completely provided. These results emphasize the importance of experimental studies to provide reliable speciation modelling in human body fluids.

Based on these backgrounds, this study focuses particularly on the experimental elucidation of the biochemical behavior of trivalent *f*-elements in the gastrointestinal tract. The digestive juices used in this study were prepared according to the Unified Bioaccessibility Method (UBM), which is developed by the Bioaccessibility Research Group of Europe (BARGE) and is based on the human physiology [28]. The chemical speciation of Ln(III)/An(III) in the digestive juices of mouth, stomach and small intestine were studied by time-resolved laser-induced fluorescence spectroscopy (TRLFS), which is a powerful method to elucidate the speciation of luminescent metal ions under lower concentrations relevant to human body [29, 30]. The luminescent cations of Cm(III) and Eu(III) were used in this study as representatives of trivalent An and Ln. The obtained spectroscopic results were also compared with thermodynamic calculations. Based on the obtained results, the possible transport of these elements through the human alimentary tract was further simulated.

2. Material and Methods

2.1 Sample preparation

Stock solutions were prepared by dissolving the solid of each inorganic (NaCl, KCl, KHCO₃, NaH₂PO₄, Na₂SO₄, KSCN, CaCl₂, NH₄Cl, NaHCO₃, KH₂PO₄ and MgCl₂ from Merck, Roth or Riedel-de Haen) or organic compound (urea, uric acid, glucose, glucosamine hydrochloride and glucuronic acid from Acros, Fluka or Calbiochem) in deionized water. All chemicals were p.a. grade with > 99 % purity. The organic samples were freshly prepared immediately before use. The proteins (amylase, pancreatin and trypsin from porcine pancreas, mucin and pepsin from porcine stomach lining, lipase from porcine pancreas Type II and bovine bile from Sigma, Roth or Applichem) were added as solids.

A stock solution of Eu(III) was prepared by dissolving EuCl₃ · 6H₂O (Sigma, 99.9 %) into deionized water, while a stock solution of Cm(III) (²⁴⁸Cm in 1 M HClO₄) was supplied from Oak Ridge National Laboratory, U.S. Department of Energy office of Basic Energy Science.

2.2 Preparation of digestive juices

The UBM protocol [28] simulates mouth, stomach and small intestine as compartments of the gastrointestinal tract. The large intestine is not included in the *in vitro* model of this study, as the main part of digestion and absorption of nutrients occur primarily in the small intestine [8]. The composition and concentration of the synthetic digestive juices are summarized in Table 1. Most of these values have been adopted from the UBM protocol though some additional modifications have been made because the UBM-based model was developed for bioavailability tests of contaminants and not for chemical speciation studies. More concretely, CaCl₂ and KHCO₃ were introduced in saliva. The saliva glands secrete a high amount of potassium and carbonate ions, which are, however, not considered in the UBM protocol. Therefore KHCO₃ was selected to simulate the large quantities of potassium and carbonate ions containing in saliva, the concentration was adapted from Hur et al. [31, 32]. Calcium ions are required as a cofactor for the enzyme α -amylase activity. The concentrations of CaCl₂ and α -amylase were modified according to the data collected for human saliva [33]. The mucin concentration was also varied from 0.05 to 0.5 mg/mL [34]. The bovine serum albumin (BSA) is expected to be a potential binding partner for Ln(III) and An(III) [35]. In fact, it occurs primarily in the blood plasma, not in the digestive system. Therefore, BSA was removed and the pancreatic enzyme trypsin was added instead as a surrogate of other proteins in the digestive juices [36, 37].

The prepared artificial digestive juices were further spiked with 1×10^{-5} M Eu(III) or 3×10^{-7} M Cm(III). In order to identify major binding partners of Eu(III)/Cm(III) in the fluids,

single components and different mixtures of components were spiked with Eu(III) and Cm(III). The pH values were adjusted with HCl or NaOH after the addition of the metal ion and were also measured prior to the spectroscopic measurements. The experiments were performed at room and body temperature (25 and 37 °C, respectively) to study potential temperature influence. For radiation safety reasons, the measurements with Cm(III) were performed in a glove box under nitrogen atmosphere, while the measurements with Eu(III) were carried out in ambient atmosphere.

2.3 Simulation of digestive system

In order to simulate the transport of the metal ions through the digestive system, the physiological conditions of the gastrointestinal compartments (stomach and intestine) were simulated according to the UBM protocol [28]. For a single transit simulation 25 mL of the synthetic saliva, gastric juice and bile fluid mixture and 50 mL of the pancreatic juice were prepared and warmed up to 37 °C. For the stomach phase, 3.3 mL of saliva was spiked with an appropriate amount of the Eu(III) and Cm(III) stock solutions to obtain the final concentrations of 1×10^{-5} M and 3×10^{-7} M, respectively. After 5-15 min, 4.95 mL of the gastric juice was added and the sample was mixed on a shaker and incubated at 37 °C for one hour. The pH value of the resultant solution was adjusted between 1.2 and 1.7. For the intestine simulation, 9.9 mL of the pancreatic juice was mixed with 3.3 mL of the bile fluid and the resultant solution was spiked with the Eu(III) stock solution to obtain the final concentration of 1×10^{-5} M. This sample was then mixed on a shaker and incubated at 37 °C for one hour. The pH of the resultant solution was adjusted between 7 and 8. The whole gastro-intestinal phase was also simulated by mixing the stomach sample with the intestine sample, which was subsequently incubated and shaken at 37 °C for four hours. The final pH value of the resultant solution was 6.3 ± 0.5 . These samples were investigated with TRLFS.

2.4 TRLFS measurements

Time-resolved laser-induced fluorescence measurements were recorded at 25 °C or 37 °C 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). A stirred cuvette holder (Flash 300™, Quantum Northwest) was used to keep the sample temperature constant during the measurements. The laser pulse energy was ranged between 1 and 3 mJ, which was

monitored using a photodiode. The emission spectra were detected by an optical multi-channel analyzer-system, consisting of a monochromator (Oriel MS 257), a spectrograph (300 or 1200 lines per mm grating) and an ICCD camera (Andor iStar). All components were purchased from the Lot-Oriel Group.

Steady-state- and time-dependent emission spectra of Eu(III) and Cm(III) were recorded from 570-650 nm (1200 lines per mm grating) and 470-810 nm (300 lines per mm grating), respectively. For all measurements a constant time window of 1 ms was applied and an excitation wavelength of 394 nm and 396 nm was applied for Eu(III) and Cm(III), respectively. The emission decay was monitored with delay time steps between 15 and 100 μ s.

The major emission transitions for Eu(III) are $^5D_0 \rightarrow ^7F_0$, $^5D_0 \rightarrow ^7F_1$ and $^5D_0 \rightarrow ^7F_2$. The $^5D_0 \rightarrow ^7F_0$ emission (570-585 nm) is a forbidden transition with a weak intensity and occurs only for Eu(III) complexes with a low symmetry. The $^5D_0 \rightarrow ^7F_1$ emission band (585-600 nm) shows a stronger intensity, which is largely independent from the coordination environment of the metal ion. For this reason the $^5D_0 \rightarrow ^7F_1$ transition is used to normalize the luminescence spectra of Eu(III). The intensity of the $^5D_0 \rightarrow ^7F_2$ emission band (610-630 nm) is strongly influenced by the coordination environment (e.g. symmetry or complexation with ligands) and, hence, it is called as a hypersensitive transition [38-40]. For the transitions of Cm(III), only a single asymmetric emission band is observed due to the $^6D_{7/2} \rightarrow ^8S_{7/2}$ transition (593.8 nm), which shows a red shift depending on the coordination environment. The lifetimes of luminescence decay also provide us information about the first coordination shell of the metal ion. For instance, the replacement of water molecules with other ligands generally results in an increase of luminescence lifetime due to the quenching effect of water molecules [29, 39].

The acquired luminescence spectra were analyzed using OriginPro9.1 (OriginLab Corporation). The Eu(III) spectra were normalized to the peak area of the $^5D_0 \rightarrow ^7F_1$ emission band, while the Cm(III) spectra were normalized to the entire peak area. The luminescence lifetimes were calculated by using following equation:

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

Equation 1 describes the exponential decay of luminescence intensity with $E(t)$ as total luminescence intensity at time t , E_i as luminescence intensity of species i at time $t = 0$ and τ_i as the corresponding luminescence lifetime. $E(t)$ was calculated by summing up the peak

areas between 550 and 725 nm. Based on the calculated lifetimes (in ms), the number of water molecules in the first coordination shell can be estimated by using the following equations from Horrocks and Kimura et al. [41-43].

$$n(H_2O) \pm 0.5 = 1.07/\tau - 0.62 \quad \text{for Eu(III)} \quad (2)$$

$$n(H_2O) \pm 0.5 = 0.65/\tau - 0.88 \quad \text{for Cm(III)} \quad (3)$$

The luminescence lifetime of the pure Eu(III) aquo complex is reported to be 110 ± 4 μ s, while that of the Cm(III) aquo complex is 65 ± 2 μ s. These lifetimes correspond to approximately 9 water molecules in the first coordination shells of the metal ions [41-43].

2.5 Linear combination fitting (LCF)

The Eu(III) luminescence spectra acquired for the single digestive juices (saliva, gastric juice, pancreatic juice and bile fluid) and their mixtures (stomach, small intestine and whole digestive system) were further analyzed by a linear combination fitting (LCF) based on the pre-selected reference spectra representing the major components in the digestive juices. The selection process of reference spectra is described in detail in the Supp. Inf. The data analysis was performed by using the Solver module on MS-Excel.

2.6 Thermodynamic modelling

Speciation calculation based on thermodynamic data was performed with Hydra/Medusa [44]. The body fluid composition and the stability constants used for the modelling are listed in Table S1 (Supp. Inf.).

3. Results and Discussion

3.1 TRLFS measurements of Eu(III) in saliva, gastric juice, pancreatic juice and bile fluid

Our experiments were performed at 25 (room temperature) and 37 °C (body temperature) to investigate possible temperature influence on complexation. The measured luminescence spectra showed, however, no significant differences between these temperatures (Supp. Inf.

Figure S3). Hence, all the measurements described below were performed at room temperature. In general, we observed two major lifetimes (shorter- and longer lifetimes) both for Eu(III) and Cm(III) TRLFS measurements. However, as discussed below, each of these observed lifetimes is found to consist of different species and, hence, it is practically difficult to derive detailed speciation information directly from the luminescence lifetime data.

The luminescence spectra of Eu(III) in the digestive juices (saliva, gastric juice, pancreatic juice and bile fluid) are shown in Figure 1 (black lines). Except for the spectrum in the gastric juice, all the spectra show an intense $^5D_0 \rightarrow ^7F_2$ emission band (610-630 nm) and the symmetry forbidden $^5D_0 \rightarrow ^7F_0$ emission band at ~580 nm, especially for the pancreatic juice and the bile fluid. This indicates the complexation of Eu(III) with the components of the saliva, pancreatic juice and the bile. All the digestive juices except for the gastric juice had the circumneutral pH of 6 to 8, while the pH of the gastric juice was 1 (Table 1). The influence of this pH difference is visible not only in the shape of TRLFS spectra but also in the calculated spectral parameters (Table 2). That is, the lifetime of the Eu(III) luminescence decays bi-exponentially (except in gastric juice), indicating the formation of at least two different species of Eu(III) in the digestive juices. The Eu(III) luminescence in the gastric juice shows a mono-exponential decay with a single lifetime ($\tau = 116 \pm 10 \mu s$) corresponding to the pure aquo ion of Eu(III) ($\tau = 111 \pm 10 \mu s$) with 9 water molecules in its first coordination shell, which is reasonable when the acidic condition of the gastric juice is taken into account. The Eu(III) luminescence in the pancreatic juice and the bile fluid shows longer lifetimes of $\tau = 224 \pm 10 / 713 \pm 23 (\tau_1 / \tau_2)$ and $231 \pm 13 / 687 \pm 27 \mu s$, respectively, which correspond to 4 and 1 water molecules in the first coordination shell of Eu(III). The lifetimes in the saliva are shorter with $\tau = 152 \pm 11 / 458 \pm 15 \mu s$, corresponding to 6 and 2 water molecules in the first coordination shell.

3.2 Speciation of Eu(III) in saliva, gastric juice, pancreatic juice and bile fluid

The acquired luminescence spectra of Eu(III) in the digestive juices were further analyzed by linear combination fitting (LCF) based on the luminescence spectra of major single components of the digestive juices, in order to identify important binding partners for Eu(III) and their fractions. The results of LCF analysis on the Eu(III) spectra in the pure digestive juices are given in Figure 1. The species distributions estimated from the LCF analysis are summarized in Figure 2-left. The components shown in Figure 2 are found to be significant components among all the possible components in the digestive juices to reproduce the

acquired experimental spectra, according to the selection criteria described in detail in the Supp. Inf.

Saliva: The LCF analysis on the experimental data indicates that the Eu(III) speciation in saliva is primarily dominated by inorganic components constituting a ternary complex, consisting of Eu, carbonate, phosphate and additionally calcium as a counter ion (named in analogy to our previous study “Eu/carb/phos(+Ca)”) (Figure 2) [24]. The attempt to fit the luminescence spectrum of saliva with only binary inorganic Eu(III) species (Eu/carb, Eu/phos) resulted in unsatisfactory residuals. However, the introduction of the ternary inorganic complex Eu/carb/phos(+Ca) resulted in a strong reduction of the residuals, improving fitting results. The addition of binary Eu(III) complexes with thiocyanate (Eu/SCN) and hydrogen carbonate (Eu/HCO₃) further improved the LCF results (Supp. Inf. Figure S4). The contributions of the proteins α -amylase and mucin appear to be insignificant on the Eu(III) speciation in saliva, as the inclusion of protein species on the LCF analysis did not improve the fitting results. Our recent study has indicated, however, a significant contribution of α -amylase and a minor contribution of mucin on the Eu(III)/Cm(III) speciation in natural human saliva [24]. Generally, the chemical speciation in a system depends strongly on the concentrations of the components. The discrepancy between this study and the previous one could be explained by different concentrations of inorganic components. That is, the concentrations of carbonate and phosphate in the simulated body fluids used in this study (15 mM) are more than three times higher than the concentrations found in natural saliva samples (4 mM) [24]. The higher concentration of these strong anionic ligands definitely enhances the complexation of Eu(III) with these inorganic components and eventually suppresses the complexation with the proteins.

The composition of natural human saliva varies significantly depending on individuals [33]. Hence, the synthetic saliva should be considered as a simple representative of human saliva, but not as the complete reproduction covering the diversity of chemical composition of human saliva.

Gastric juice: Because of the acidic condition of pH = 1, no significant complexation with inorganic or organic ligands is expected in the gastric juice, which is consistent with the TRIFS results. The LCF results suggest that 95 % of the Eu(III) species exist as a pure aquo complex. The remaining 5 % are difficult to identify, but could possibly be the species with organic components (e.g., mucin or pepsin) (Figure 2-left).

Pancreatic juice: In general, chemical speciation is not only concentration dependent but also pH dependent. As compared to the gastric juice, there is a huge increase of the pH to ~7 in the

pancreatic juice. Additionally, pancreatic juice contains a wider variety of digestive enzymes (more than 20) than those in saliva and gastric juices [46]. Consequently, the Eu(III) speciation in the pancreatic juice is dominated by organic species rather than inorganic ones that were found to be dominant in saliva. The LCF analysis suggests that 60 % of Eu(III) species are forming complexes with mucin, while the ternary inorganic complex Eu/carb/phos(+Ca) still shows a moderate contribution (27 %) on the Eu(III) complexation with the additional contribution of binary Eu/carbonate species (13 %) (Figure 2-left). Given the high carbonate concentration in the pancreatic juice, the fraction of Eu/carbonate species would be expected to be higher than the observed value. This indicates that the protein mucin seems to have a higher affinity to Eu(III) than carbonates. This is further confirmed by the fact that the luminescence spectra of Eu(III) with mucin are not significantly affected by the presence of the physiological concentration of carbonate (Figures S1 and S5).

Bile fluid: The LCF analysis on the TRLFS results suggest that 45 % of Eu(III) species are forming the ternary inorganic complex Eu/carb/phos(+Ca) in the bile fluid, and the rest of 55 % are forming complexes with the bile secretion (Figure 2-left). Bile represents a complicated system with various compounds such as bile salts and alcohols, cholesterol, metal ions, metabolic products, phospholipids, etc. [47]. Therefore, many components of the bile, such as phospholipids, could potentially interact with Eu(III). The study by Li et al. [48] reported the effect of Ln(III) (La(III), Gd(III) and Yb(III)) on multilamellar liposomes, indicating that the interaction between the metal ion and liposomes becomes stronger with a decrease of the ionic radius of Ln(III). Therefore, the interaction of Eu(III) with phospholipids, a major component of liposomes, could be expected in the pure bile fluid. This interaction can be, however, suppressed by the presence of stronger ligands, such as EDTA [48], suggesting that the Eu(III) species with some bile components (e.g. phospholipids) could be dissociated when the relevant species are exposed to the pancreatic juice possessing stronger ligands such as the protein mucin.

3.3 Speciation of Eu(III) in the stomach, small intestine and the whole digestive system

According to the actual human digestive tract, single digestive juices were blended to simulate the whole digestive system. First saliva and gastric juice were mixed to simulate the condition in the stomach, which is further followed by blending pancreatic juice and bile fluid to simulate the physiological condition in the small intestine. Finally, the simulated mixtures for stomach and small intestine were mixed to simulate the whole digestive system. The

luminescence spectra collected in these simulated digestive systems are shown in Figure 3, and the results of the LCF analysis are summarized in Figure 4-left.

Stomach: In our digestive process, a mixture of food and saliva in the mouth, which is so-called *bolus*, passes through the esophagus and is eventually transported into the stomach [49]. The pH of pure gastric juice is 0.9-1.0, while the pH of the saliva-gastric juice mixture is between 1.2 and 1.7. This indicates a slight but significant increase in pH when the ingested substances in the mouth are transported into the stomach with saliva, surely altering the chemical speciation. The Eu(III) species distribution derived from the LCF analysis on the TRLFs data suggests that, as compared with the speciation in the pure gastric juice, the Eu(III) speciation in the stomach is more intricate. The contributions of the mucin and inorganic species are found to be low, accounting for 12 % of the total fraction. Additionally, the formation of the pure aquo species is suppressed in the stomach (61 %), where the fraction in the pure gastric juice is 95 %. Instead, a new Eu(III) species with pepsin (26 %), the specific digestion enzyme of the stomach, was detected in the stomach [50]. The direct interaction between Eu(III) and pepsin was also confirmed by another TRLFs measurement on the sample containing only pepsin without other ligands (Supp. Inf. Figure S2).

Small intestine: In the small intestine, two bio fluids are co-existing: the pancreatic juice and the bile fluid. The former one is secreted by the pancreas and then transported into the upper part of the small intestine (duodenum), while the latter one is produced in the liver and stored in the gallbladder until requirement [31]. The mixture of these two fluids has the pH ranging between 7 and 8. As shown in Figure 4-left, the results of LCF analysis indicates a major contribution of the Eu(III) species with mucin (66 %) in this fluid mixture. The relevant species is also found in the pure pancreatic juice. The species with the bile components are found to be minor (6 %). However, the secreted bile fluid potentially contains mucin as a component [47] and, hence, the calculated fraction of the bile species could be possibly added to the fraction of the mucin species. Some inorganic complexes were also identified but with a minor contribution to the total fraction.

Whole digestive system: Combining all the four bio fluids results in a pH of 6.3 ± 0.5 . The results of LCF analysis suggests two major species, one with mucin (42 %) and the ternary inorganic complex Eu/carb/phos(+Ca) (43 %). Due to the high carbonate concentration originating from the pancreatic juice and the bile fluid, binary Eu/carbonate complexes are also formed.

The LCF results on the digestive fluids suggest that two or three independent Eu(III) species are present in the fluids, while their luminescence decay was either mono- or bi-exponential. Hence, the obtained lifetimes data should be considered rather as average values for multiple species, not for a specific single species. However, the obtained lifetime data shows some general trends, which support the LCF results at least qualitatively. In saliva, at circumneutral pH, the luminescence lifetimes indicate the replacement of 3 to 7 water molecules with other ligands in the first coordination shell. This points to a strong complexation with saliva components. In gastric juice and the stomach mixture at acidic pH, the first coordination shell of Eu(III) retains 8.6 and 8.2 water molecules, respectively, which nearly equal to the pure hydration state (i.e. 9 water molecules in the first coordination shell). When the pH returns to circumneutral in the intestine with the pancreatic juice and the bile fluid as well as the whole digestive juice, the lifetimes data indicate almost a complete dehydration in the first coordination shell of Eu(III) and the replacement with other ligands.

3.4 TRLFS measurements of Cm(III)

Based on the results with Eu(III), the sample media used for Cm(III) experiments were pre-selected (i.e., mouth, stomach, small intestine and the whole digestive juice). The TRLFS spectra of Cm(III) in the selected gastrointestinal fluids are shown in Figure 5, together with the reference spectra of the pure aquo Cm(III) species and the complex with mucin. The obtained spectral parameters are summarized in Table 3. As compared with the reference spectrum of the pure aquo species, the spectrum in saliva shows a red shift, indicating the interaction of Cm(III) with other ligands in saliva. The luminescence lifetime of Cm(III) in saliva decayed bi-exponentially, suggesting at least two different Cm(III) species in this medium. The calculated lifetimes of 98 ± 15 and 346 ± 18 μ s correspond to 6 and 1 water molecules in the first coordination shell, respectively. Because of the acidic condition, the hydration of Cm(III) is found to be enhanced in the stomach, which is in line with the Eu(III) results. The calculated luminescence lifetime of 68 ± 5 μ s for the stomach sample is well comparable to that of the pure aquo ion (65 ± 2 μ s, coordination number = 9) [42]. The spectrum in the mixture of the pancreatic juice and the bile fluid shows again a red shift. The luminescence lifetimes also decayed bi-exponentially, however, the lifetimes themselves are longer than those calculated for the mouth sample. The calculated lifetimes are 129 ± 9 and 380 ± 12 μ s, corresponding to 4 and 1 water molecules in the first coordination shell,

respectively. The spectrum in the mixture of all the digestive juices also shows a red shift with similar spectral parameters.

In the measurements with Eu(III), the protein mucin was found to be an important ligand. The TRLFS spectrum of Cm(III) with mucin also shows a significant red shift and its lifetime decays bi-exponentially. Similar trends are also observed in the intestine and the whole digestive system samples. Hence, we conclude that mucin is an important organic component not only for the Eu(III) speciation but also for the Cm(III) one.

The Eu(III) luminescence spectra measured in this study originate from the $^5D_0 \rightarrow ^7F_1$ and $^5D_0 \rightarrow ^7F_2$ transitions, the intensity ratios and splitting patterns of which vary significantly with different coordination geometry around the Eu(III) center. In contrast, the Cm(III) luminescence spectra, which originate from the $^6D_{7/2} \rightarrow ^8S_{7/2}$ transition, are less sensitive to the change in coordination geometry (i.e. speciation) [24]. Hence, the TRLFS for Eu(III) is more sensitive and more powerful tool for chemical speciation than that for Cm(III).

To summarize the results described thus far, we have simulated the *in vivo* transition of trivalent *f*-elements (An(III)/Ln(III)) from the stomach to the small intestine. Because of the acidic condition, the speciation of An(III)/Ln(III) in the stomach is dominated by the pure aquo ion with a significant contribution of the complex with pepsin. When the An(III)/Ln(III) species are further transported into the small intestine, they are subject to an increase in pH, enhancing the interaction with the protein mucin as well as the formation of a ternary inorganic complex (An or Ln/carb/phos(+Ca)). Similar speciation with mucin and the ternary inorganic complex is also expected in the mixture of the whole digestive juices.

3.5 Comparison of spectroscopic measurements with thermodynamic calculations

Chemical speciation can be also simulated by thermodynamic models. The first thermodynamic speciation calculation for An/Ln in the human gastrointestinal tract was published by Webb et al. in 1998 [26, 27]. The simulation was primarily based on inorganic components and only a limited number of organic compounds, such as citrate, lactate or amino acids, were included as possible binding partners. Bio-macromolecules such as digestive proteins were not included in the simulation, simply because of the limited availability of thermodynamic data at that time. They simulated the speciation of Eu(III) and Am(III) in saliva and gastric juice, as well as those of Gd(III) and Cm(III) in the small intestine (pancreatic juice and bile fluid). Their speciation calculations differ significantly

from our experimentally determined speciation. For instance, they reported that the speciation in the mouth is dominated by hydrogen carbonate or citrate for Eu(III) and Am(III), respectively, while the pure aquo complexes are the major species in the stomach. In the small intestine, the speciation is governed by the phosphate and carbonate and hydroxide complexes for Gd(III) and Cm(III), respectively. These results are inconsistent with our experimental results in this study. It should be also noted that they calculated different speciation for Ln and An contrary to the expected chemical similarity.

Based on the recent stability constants, we have also performed thermodynamic modelling for the Eu(III) speciation. The results are summarized in Figures 2 and 4. The results are, however, also not consistent with our experimental data derived from the TRLFS measurements. This is, again, because of the lack of available thermodynamic data particularly for larger organic components (e.g. mucin and pepsin) as well as multi-component inorganic species (e.g. ternary complex with carbonate, phosphate and calcium). This, together with the discrepancy found in the pioneer study by Webb et al, clearly highlights the importance of further determining the stability constants for organic- and multi-component species for the reliable speciation calculations. Nevertheless, the thermodynamic modelling could be still informative especially when the system is dominated by simple inorganic species. For instance, there is a good agreement between the experimental data and the thermodynamic modelling for the saliva and gastric juice systems, which are dominated by inorganic species and the pure aquo ion, respectively (Figure 2). The thermodynamic calculations could be also useful to have a detailed overview on the inorganic part of the whole speciation, particularly in case some species are not distinguishable experimentally.

Furthermore, the stoichiometry and the protonation behavior of the ternary complex Eu/carb/phos(+Ca) is unknown yet. The thermodynamically determined distribution of Eu/carbonate and Eu/phosphate species (e.g. in small intestine and whole digestive system, Figure 4-right) can help characterizing the exact composition of this ternary complex as it has been performed for saliva samples [24]. There, based on thermodynamic calculation, a 1:1:2 (Eu:carb:phos) complex with deprotonated carbonate species (CO_3^{2-}) is suggested. However, the protonation of phosphate ligands is sensitive to pH and ligand concentration and, hence, special cares are required to understand the protonation behavior in the respective body fluids.

3.6 Absorption of trivalent f-elements in the gastrointestinal tract

Based on the results obtained in this study, we could further interpret possible *in vivo* behavior of trivalent *f*-elements in the human gastrointestinal tract, their interaction with digestive enzymes and proteins and their potential absorption mechanisms. Our results show that the speciation of trivalent *f*-elements is dominated by inorganic phosphate/carbonate species in the first contact medium of saliva in the mouth. The speciation changes mainly into the pure aquo ions when they move into the stomach, where the interaction with pepsin, the main gastric enzyme, becomes also significant. Pepsin has a negative charge at a wide pH range between 1 and 5 [51, 52] and its complexation with other metal ions (Al(III), Ni(II) and Cu(II)) was also reported. The interaction between pepsin and these metal ions eventually leads to the increase of pepsin activity with increasing the concentration of metal ions [52-54]. Other studies based on *in vitro* digestion experiments suggested the formation of metal-protein complexes between the heavy metals (e.g., Mn, Pb, Zn) dissolved from soil and digestive enzymes (e.g., pepsin or trypsin). As a consequence, the dissolution of heavy metal ions from soil to digestive juices is facilitated. These findings could be applicable also to the solid food containing (radio-)toxic heavy metal ions. That is, the presence of digestive enzymes (e.g. pepsin) and/or proteins could facilitate the dissolution of heavy metal ions injected with the solid food into the digestive fluid, potentially playing a vital role in the fate of heavy metal ions in the digestive system [55, 56].

The aquo ions formed in the stomach are eventually transformed primarily to the organic species with mucins when they move into the small intestine. The species with pepsin are likely to convert also into other species, because pepsin is expected to be hydrolyzed at the intestinal pH [57]. In the small intestine, the majority of digestion and resorption processes of nutrients take place. It has been reported that the absorption of transuranium elements (Np, Pu, Am, Cm and Bk) also occurs mainly in the small intestine [2, 49]. Hence, the chemical species of trivalent *f*-elements with mucins would be the vital species that potentially influence the accumulation and incorporation of these (radio-)toxic heavy metal ions in the human body. Mucins are high molecular weight glycoproteins comprising the main components of the human mucosa. The presence of mucins in the mucosa allows the formation of viscous membrane layers in the gastrointestinal tract, providing a vital function to prevent drugs, bacteria, viruses or other chemical substances including heavy metal ions from the absorption [58, 59]. Consequently, our present results demonstrating the strong complexation between trivalent *f*-elements and mucins would be positive news from the toxicological viewpoint, as the presence of mucins in the small intestine would suppress the absorption of these elements into the bloodstream and eventually help their efficient

excretion. In order to further investigate the *in vivo* functions of mucins towards (radio-)toxic elements including *f*-elements, further experiments, such as the determination of the stability constants or speciation by mass spectrometry, etc., are currently being performed.

Reliable information on chemical speciation can also help to develop the decontamination treatments required to reduce health risks after the ingestion of contaminants into the human body. The common treatment for actinides contamination uses DTPA (Diethylenetriaminepentaacetic acid or (carboxymethylimino)bis(ethylenenitrilo)tetraacetic acid) as a chelating agent to facilitate the excretion by forming extractable complexes with actinides. The oral activity of DTPA is, however, expected to be low and it can interact only moderately with trivalent actinides after oral administration. In the last decades, several new chelating ligands have been developed for the decontamination treatment of actinides contaminants. Cyclic hydroxypyridinonates (HOPO) are some of those ligands inspired by natural occurring siderophores [60]. Siderophores are highly selective microbial iron transporter with catechol groups as binding units. The promising candidates in this type of ligands are 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) [61]. In contrast to DTPA, these ligands remain active in the oral conditions and they are reported to be effective for trivalent and tetravalent actinides [60, 61]. The present study shows that mucin is playing an important role in the speciation of trivalent *f*-elements in the gastrointestinal tract. Assuming that the siderophore-based HOPO ligands could keep their activity towards heavy metal ions even in the gastrointestinal tract, the HOPO ligands could form soluble complexes with trivalent *f*-elements in addition to the complexes with mucin, further facilitating the efficient excretion of these heavy metals in a cooperative manner. This also highlights the importance of additional studies focusing on the competitive complexation of toxic metal ions between the biomacromolecules/proteins and decontamination reagents under physiological conditions.

4. Conclusions

We reported the first experimental study focusing on the chemical speciation of a trivalent lanthanide (Eu(III)) and an actinide (Cm(III)) in the human gastrointestinal tract media including artificial digestive juices (saliva, gastric juice, pancreatic juice and bile) and the simulated whole digestive media as described in the UBM protocol [28]. The results suggest that the inorganic species, such as phosphate/carbonate or pure aquo ions, are dominant in the mouth and stomach, while the organic complex with the enzyme pepsin plays a significant

role in the stomach. The organic species with the protein mucin are the most significant species in the intestinal tract including the small intestine where most of the digestion and absorption of nutrients occur. The strong complexation ability of mucins towards the trivalent *f*-elements could suppress the absorption of these elements in the human body and would facilitate their excretion. This study also highlights the importance of the experimental determination of the stability constants and chemical speciation particularly for the systems including biomacromolecules and/or multi-component species, for which the current thermodynamic database is incomplete and may not be reliable.

The results obtained in this study could also help to develop the decontamination measurements or to assess the health risks when these radionuclides are incorporated in the human digestive system. Speciation analyses are very important for a better understanding of the biochemical behavior of trivalent *f*-elements. All biological steps as absorption, transport, distribution in organs or excretion depend on the chemical speciation with biological ligands. Reliable information on the chemical speciation can support the development of effective decontamination treatments that are required to reduce health consequences after the incorporation of radionuclides [62].

Abbreviations

An(III), trivalent actinides; BARGE, Bioaccessibility Research Group of Europe; DTPA, Diethylenetriaminepentaacetic acid; HOPO, hydroxypyridinonates; ICCD, Intensified Charge-Coupled Device; LCF, linear combination fitting; Ln(III), trivalent lanthanides; Nd:YAG-OPO, neodymium-doped yttrium aluminum garnet optical parametric oscillator; TRIFS, time-resolved laser-induced fluorescence spectroscopy; UBM, Unified Bioaccessibility Method.

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Tables

Table 1: Compositions and concentrations of the synthetic digestion juices used in this study taken from UBM protocol [28] unless otherwise marked.

| | Saliva | | Gastric Juice | | Pancreatic Juice | | Bile Fluid | |
|------------------|----------------------------------|------------------|----------------------------------|-----------|---------------------------------|--------------------|--------------------|-----------|
| inorganic | KCl | 24.0 mM | NaCl | 94.2 mM | NaCl | 234.0 mM | NaCl | 180.0 mM |
| | KHCO ₃ | 15.0 mM [32, 33] | KCl | 22.1 mM | NaHCO ₃ | 133.5 mM | NaHCO ₃ | 137.7 mM |
| | NaH ₂ PO ₄ | 14.8 mM | NH ₄ Cl | 11.4 mM | KCl | 15.1 mM | KCl | 10.1 mM |
| | NaCl | 10.2 mM | CaCl ₂ | 5.4 mM | CaCl ₂ | 1.4 mM | CaCl ₂ | 1.5 mM |
| | Na ₂ SO ₄ | 8.0 mM | NaH ₂ PO ₄ | 3.9 mM | KH ₂ PO ₄ | 1.2 mM | | |
| | KSCN | 4.1 mM | | | MgCl ₂ | 0.5 mM | | |
| | CaCl ₂ | 1.0 mM [33] | | | | | | |
| organic | urea | 6.7 mM | glucose | 7.2 mM | urea | 3.3 mM | urea | 8.3 mM |
| | uric acid | 0.1 mM | glucosamine hydrochloride | 3.1 mM | | | | |
| | | | urea | 2.8 mM | | | | |
| | | | glucuronic acid | 0.2 mM | | | | |
| proteins | amylase | 1.0 mg/mL | mucin | 3.0 mg/mL | mucin | 3.0 mg/mL | bovine bile | 6.0 mg/mL |
| | mucin | 0.5 mg/mL [34] | pepsin | 1.0 mg/mL | pancreatin | 3.0 mg/mL | | |
| | | | | | trypsin | 1.0 mg/mL [36, 37] | | |
| | | | | | lipase | 0.5 mg/mL | | |
| pH | 6.5±0.5 | | 1.0±0.1 | | 7.4±0.2 | | 8.0±0.2 | |

Table 2: Spectral parameters of Eu(III) luminescence in artificial digestive juices, simulated digestive systems and solutions of major single components.

| Sample | pH | Exponential decay manner ^a | Luminescence lifetime and estimated hydration number ^b | | | |
|------------------------------------|-----|---------------------------------------|---|---------------------------|---------------------|---------------------------|
| | | | τ_1 (μ s) | $n_1(\text{H}_2\text{O})$ | τ_2 (μ s) | $n_2(\text{H}_2\text{O})$ |
| Eu ³⁺ (aq) | 1.0 | mono | 111 \pm 10 | 9.0 | | |
| Artificial digestive juices | | | | | | |
| Saliva | 6.7 | bi | 152 \pm 11 | 6.4 | 458 \pm 15 | 1.7 |
| Gastric juice | 1.0 | mono | 116 \pm 10 | 8.6 | | |
| Pancreatic juice | 7.6 | bi | 224 \pm 10 | 4.2 | 713 \pm 23 | 0.9 |
| Bile fluid | 8.0 | bi | 231 \pm 13 | 4.0 | 687 \pm 27 | 0.9 |
| Simulated digestive system | | | | | | |
| Stomach | 1.4 | mono | 121 \pm 10 | 8.2 | | |
| Intestine | 8.1 | bi | 162 \pm 10 | 7.9 | 533 \pm 28 | 2.2 |
| Whole system | 6.8 | bi | 261 \pm 11 | 3.5 | 1299 \pm 32 | 0.2 |
| Single and mixed components | | | | | | |
| Carb | 6.9 | mono | 126 \pm 10 | 7.9 | | |
| | 8.1 | mono | 192 \pm 10 | 4.9 | | |
| SCN ⁻ | 6.8 | mono | 210 \pm 10 | 4.5 | | |
| SO ₄ ²⁻ | 6.4 | mono | 255 \pm 10 | 3.6 | | |
| Phos | 6.4 | mono | 235 \pm 10 | 3.9 | | |
| Carb/Phos+Ca | 6.8 | bi | 363 \pm 48 | 2.3 | 701 \pm 61 | 0.9 |
| Urea | 6.8 | mono | 155 \pm 10 | 6.3 | | |
| Amylase | 6.2 | bi | 412 \pm 21 | 2.0 | 794 \pm 18 | 0.7 |
| Mucin | 1.0 | mono | 119 \pm 10 | 8.4 | | |
| | 7.2 | bi | 311 \pm 16 | 2.8 | 746 \pm 16 | 0.8 |
| Pepsin | 4.5 | mono | 132 \pm 10 | 7.5 | | |
| Lipase | 6.4 | bi | 269 \pm 18 | 3.4 | 677 \pm 11 | 1.0 |
| Pancreatin | 6.0 | bi | 314 \pm 31 | 2.8 | 768 \pm 11 | 0.8 |
| Trypsin | 7.3 | bi | 204 \pm 10 | 4.6 | 458 \pm 11 | 1.7 |
| Bile | 7.8 | bi | 420 \pm 20 | 1.9 | 872 \pm 19 | 0.6 |

^a Corresponds to index i in eq. (1) ^b Number of water molecules \pm 0.5 according to eq. (2)[41, 42, 43].

Table 3: Spectral parameters of Cm(III) luminescence in artificial digestive juices, simulated digestive systems and solutions of major single components.

| Sample | pH | λ (nm) ^a | Exponential decay manner ^b | Luminescence lifetime and estimated hydration number ^c | | | |
|-----------------------------------|------|-----------------------------|---------------------------------------|---|---------------------------|---------------------|---------------------------|
| | | | | τ_1 (μ s) | $n_1(\text{H}_2\text{O})$ | τ_2 (μ s) | $n_2(\text{H}_2\text{O})$ |
| Cm ³⁺ (aq) | 2.30 | 593.8 | mono | 67 \pm 5 | 8.8 | | |
| Simulated digestive system | | | | | | | |
| Mouth | 6.4 | 603.9 | bi | 98 \pm 15 | 5.7 | 346 \pm 18 | 1.0 |
| Stomach | 1.3 | 593.5 | mono | 68 \pm 5 | 8.7 | | |
| Intestine | 7.4 | 602.6 | bi | 129 \pm 9 | 4.2 | 380 \pm 12 | 0.8 |
| Whole system | 6.8 | 603.7 | bi | 138 \pm 7 | 3.8 | 498 \pm 13 | 0.4 |
| Single components | | | | | | | |
| Mucin | 6.0 | 603.1 | bi | 81 \pm 5 | 7.2 | 259 \pm 5 | 1.6 |

^a Main emission wavelength \pm 0.1 nm.). ^b Corresponds to index i in eq. (1) ^c Number of water molecules \pm 0.5 according to eq. (3) [41, 42, 43].

Figures

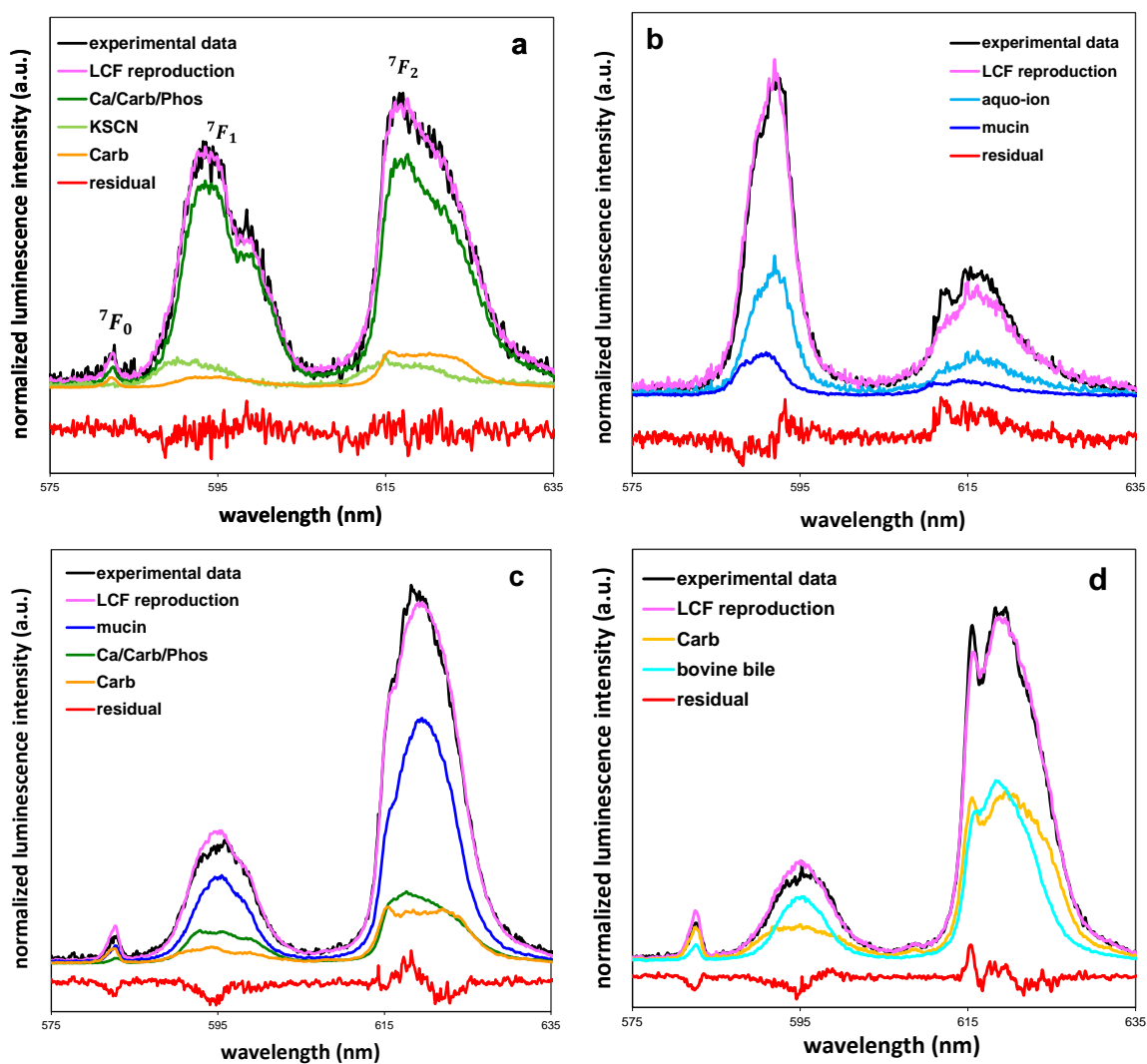


Figure 1: Luminescence spectra of 1×10^{-5} M Eu(III) in saliva (a), gastric juice (b), pancreatic juice (c) and bile fluid (d) and their linear combination fitting based on major inorganic and organic components. The $({}^5D_0 \rightarrow {}^7F_0)$, $({}^5D_0 \rightarrow {}^7F_1)$ and $({}^5D_0 \rightarrow {}^7F_2)$ transitions are marked in (a).

Based on TRLFS results

Based on thermodynamic modelling

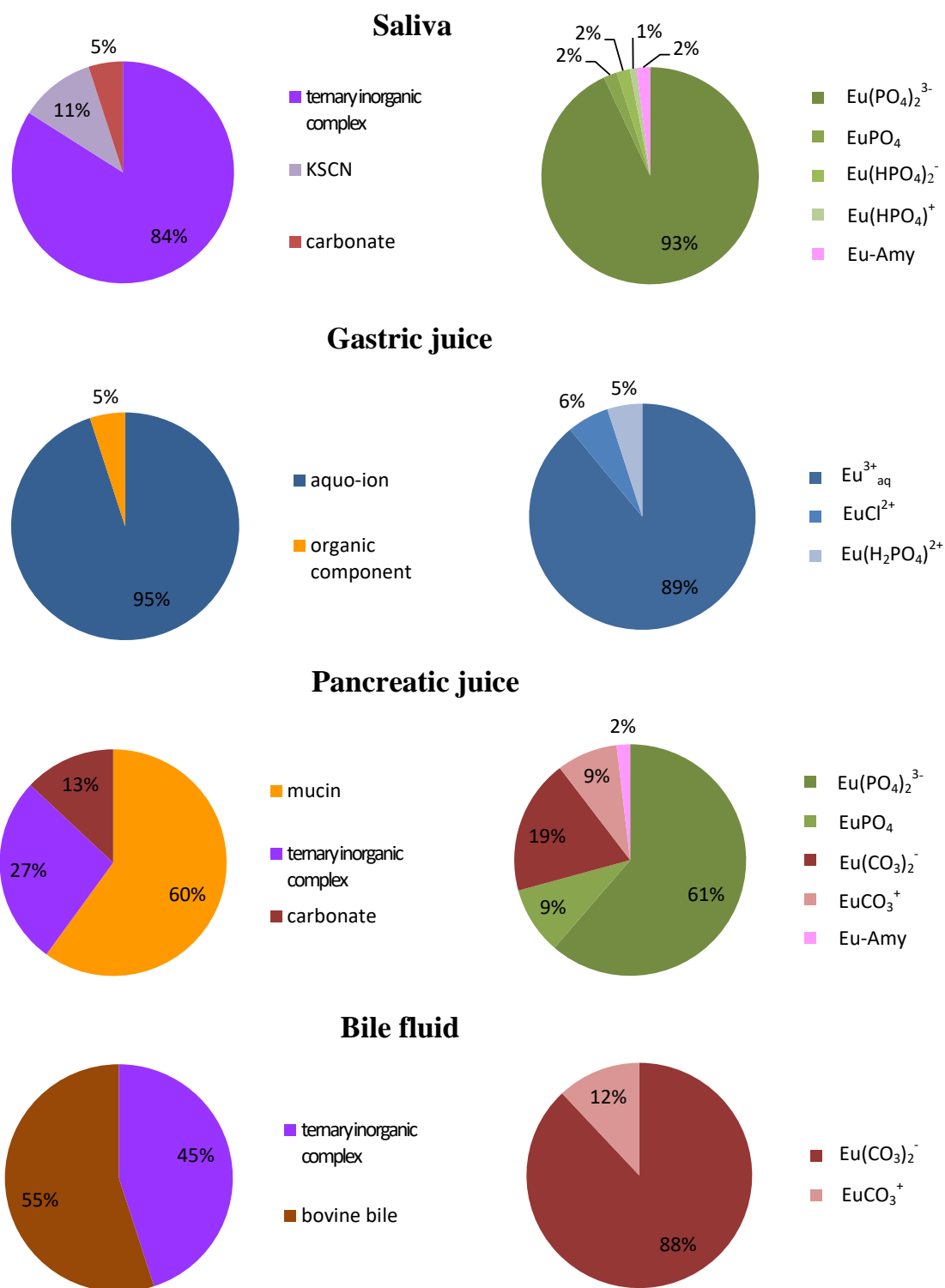


Figure 2: Species distributions of 1×10^{-5} M Eu(III) in saliva (pH 6.7), gastric juice (pH 1.0), pancreatic juice (pH 7.6) and bile fluid (pH 8.0) based on experimental data (TRLFS, left) and thermodynamic modelling (right, also see Supp. Inf. Table S1).

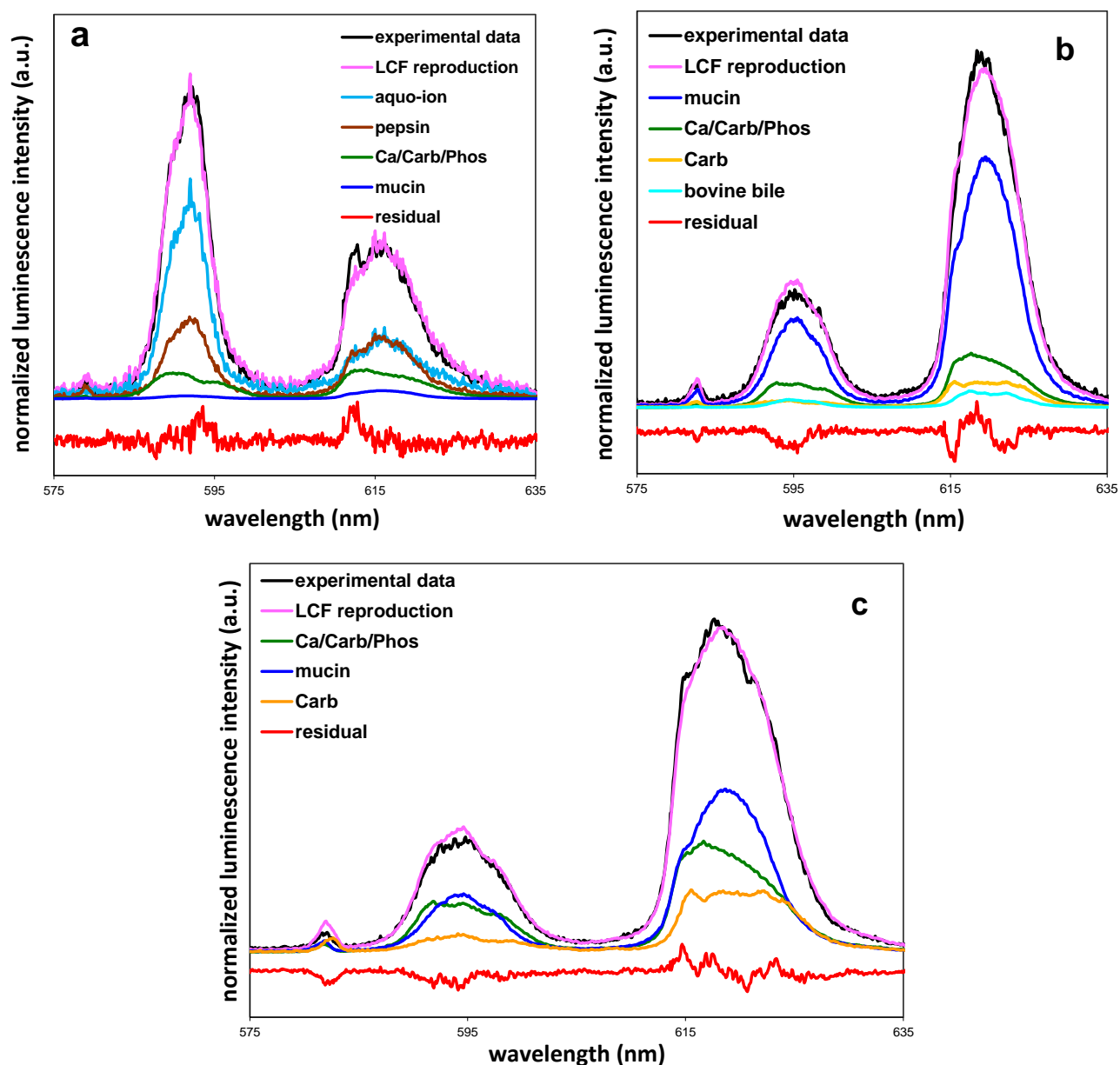
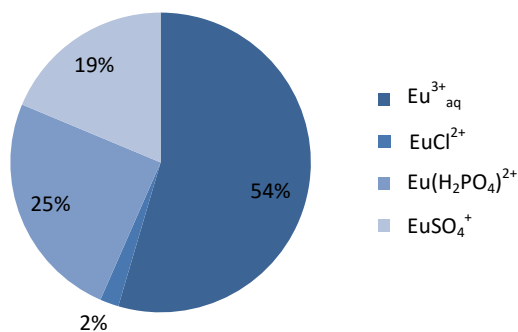
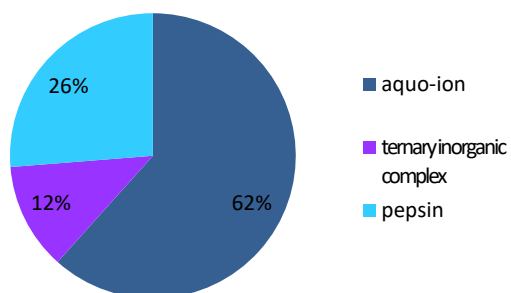


Figure 3: Luminescence spectra of Eu(III) in the simulated media of stomach (a), small intestine (b) and whole digestive system (c) and their linear combination fitting based on major inorganic and organic components.

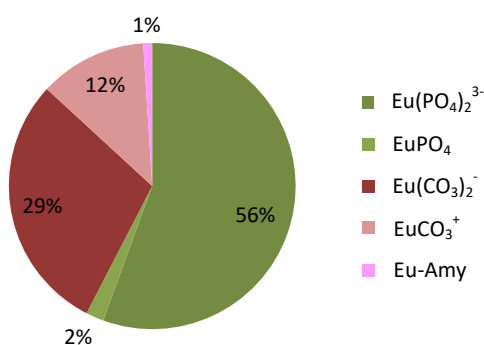
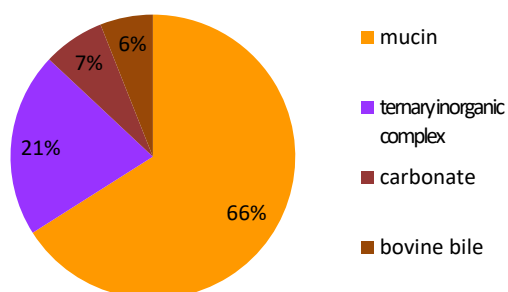
Based on TRLFS results

Based on thermodynamic modelling

Stomach



Small intestine



Whole digestive system

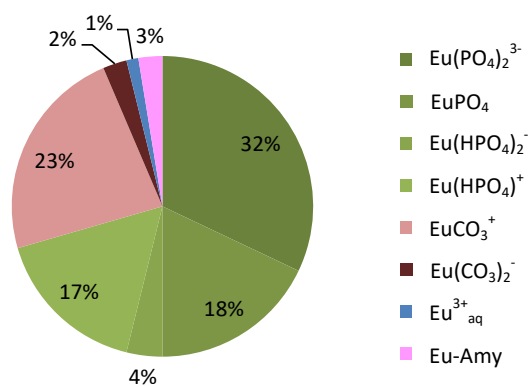
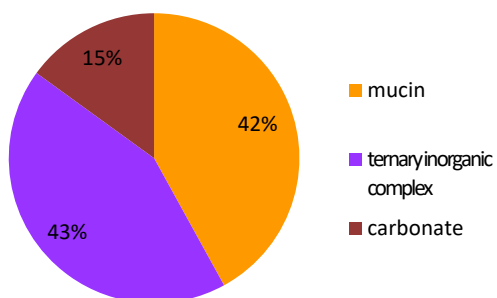


Figure 4: Species distributions of 1×10^{-5} M Eu(III) in simulated stomach (pH 1.3), small intestine (pH 7.4) and whole digestive system (pH 6.8) based on experimental data (TRLFS, left) and thermodynamic modelling (right, Supp. Inf. Table S1).

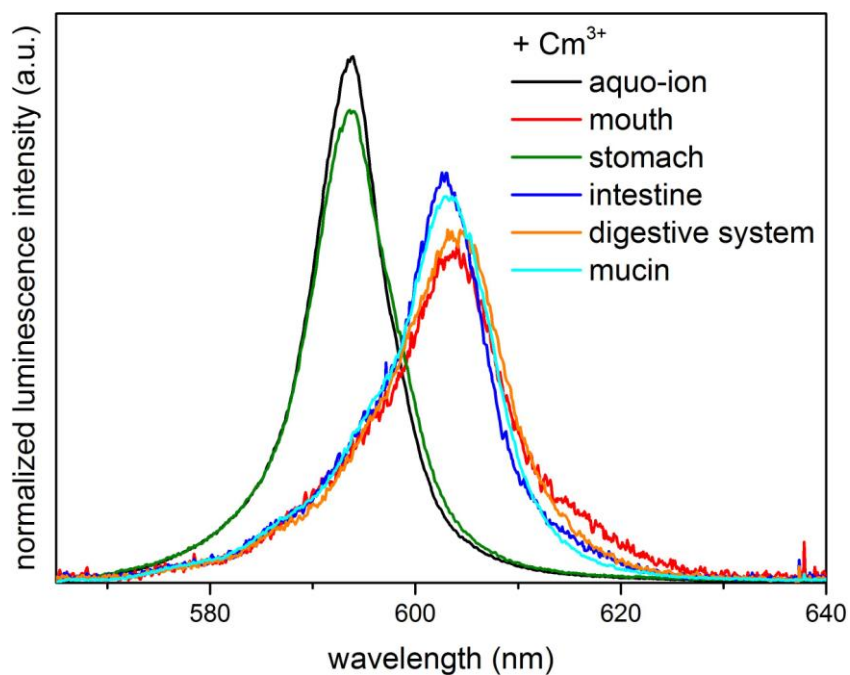


Figure 5: Luminescence spectra of Cm(III) in the simulated media of mouth, stomach and small intestine as well as the reference spectra of pure aquo complex (black data) and the species with mucin (light blue data).