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In situ luminescence analysis: a new light on monitoring calcium phosphate phase transitions†‡

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In this work, *in situ* luminescence analysis was applied for the first time for monitoring the phase transitions of calcium phosphate (CaP) and confirmed by synchrotron *in situ* X-ray diffraction in addition to *in situ* infrared spectroscopy, with simultaneous measurements of pH and ion conductivity. Applying doped Ce^{3+} and Eu^{3+} as local coordination sensors, the high sensitivity of their emission spectra upon the changes in the coordination sphere of the doped cation sites enabled to detect the formation of amorphous calcium phosphate (ACP) and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, besides their subsequent transitions to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ under real reaction conditions. Calcium phosphates are widely present in mammals and understanding their phase transitions is important to comprehend the conversion between healthy and diseased tissues. *In situ* luminescence measurements are advantageous for allowing monitoring these phase transitions in a fast and sensitive fashion also in conventional laboratories, independent of synchrotron radiation.

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1. Introduction

Efficiently monitoring the events occurring during the formation of solids in solutions including nucleation, crystal growth and formation of reaction intermediates as well as consequent phase transitions culminating in the crystallization of the final product require the application of *in situ* characterization techniques under real reaction conditions.^{1–10} Studying the mechanism of chemical reactions by removing samples from the reactor during the synthesis process and analysing them *ex situ* is rather disadvantageous for offering only snapshots of the process with very limited time resolution, besides probably influencing the sample while preparing them for the *ex situ* analysis.^{1,2} The formation and phase transitions of calcium phosphate (CaP) systems offer a critical example for

the importance of applying *in situ* techniques. Calcium phosphate derivatives are one of the main inorganic components in mammals and are widely used for producing medical implants and prosthesis.^{11,12} For instance, amorphous calcium phosphate (ACP) is found in pathological tissues like heart valve calcifications,¹¹ $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ is proposed as an intermediate in bone mineralization,¹¹ $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ is present in human dental and urinary calculi,¹³ CaHPO_4 is used in bone cements,¹⁴ while $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ is used for coating orthopaedic and dental implants.¹⁵ Therefore, understanding the formation and transition of the CaP phases is important for comprehending the transitions between healthy and diseased tissue as well as for predicting and preventing the degradation of implants and prosthesis in our bodies. In addition, studying the formation of CaP is essential for understanding the formation of amorphous pre-nucleation clusters, which is still a challenge for the currently accepted nucleation theories.^{16–18} Since the phase transitions of CaP are extremely sensitive to variations in the environmental conditions such as temperature, pH and concentration, they should be ideally studied only *in situ* under real reaction conditions, in order to not disturb the investigated processes.¹² Up to now, different powerful *in situ* techniques have been reported in the literature for monitoring the mineralization mechanisms of CaP systems, as recently summarized by Pan *et al.*¹² Some of these *in situ* techniques are *in situ* pH measurements,¹⁹ UV/Vis absorption spectroscopy,²⁰ Raman,²¹ turbidity,²² quartz crystal microbalance (QCM),²³ X-ray diffraction (XRD)²⁴ and X-ray absorption spectroscopy (XAS).²⁵ However, many of these techniques provide

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‡Electronic supplementary information (ESI) available: Detailed description of experimental setup, complementary *ex situ* XRD and SEM measurements as well as additional results of *in situ* excitation spectra, IR spectroscopy and synchrotron-based XRD experiments. See DOI: 10.1039/c7qi00172j



In this work, the ILACS approach utilizes the $5d \rightarrow 4f$ transitions of Ce^{3+} and the $4f \rightarrow 4f$ transitions of Eu^{3+} for monitoring the formation of ACP and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ as well as their conversion to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$. These processes have been confirmed here by *ex situ* and synchrotron-based *in situ* XRD, besides *in situ* measurements of pH value, ion conductivity and infrared (IR) spectroscopy. To the best of our knowledge, the application of *in situ* luminescence and IR techniques for monitoring CaP phase transitions as well as the optical properties of Ce^{3+} and Eu^{3+} -doped $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ are reported here for the first time.

For the synthesis of the different calcium phosphate phases, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (98.5+%, Merck KGaA, Darmstadt, Germany), $\text{Eu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (99.9%, ChemPur Feinchemikalien und Forschungsbedarf GmbH, Karlsruhe, Germany), $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (99.99%, Alfa Aesar GmbH & Co KG, Karlsruhe, Germany) and anhydrous $(\text{NH}_4)_2\text{HPO}_4$ (99+%, Merck KGaA, Darmstadt, Germany) have been used without further purification. The synthesis methods reported in this work consist of simplified co-precipitation techniques and the solutions have been freshly prepared for every trial, in which the $(\text{Ca}, \text{Ln})\text{:PO}_4^{3-}$ ratio was approximately 3:2. The concentrations and temperatures used specifically for each experiment are listed in Table 1. A detailed explanation of the assemblies used on experiments at the University of Kiel and at the Deutsches Elektronen-Synchrotron (DESY, Hamburg, Germany), respectively Setup A and Setup B, is supplied in the ESI.†

In a typical synthesis procedure applying Setup A (experiments 1–8, Table 1), 20 mL of an aqueous solution of diammonium hydrogen phosphate are placed inside the reactor. Subsequently, 5–10 mL of an aqueous solution of calcium and cerium or europium nitrates were added to the reactor during the first 10 minutes of the reaction. During the dosing process, the temperature was kept at 10 °C and increased after

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For instance, the *ex situ* XRD patterns presented here (Fig. S6–S8†) show a time range where the phase transitions can occur, but not the exact time. In addition, the preparation procedure of the samples removed from the reactor for the *ex situ* characterization such as quenching, washing and drying might influence the product, generating possible divergences between the *ex situ* results and the actual phenomena occurring during the reactions.² For this reason, *in situ* luminescence measurements applying 3% of doping concentration have been carried out for the coordination sensors Ce^{3+} and Eu^{3+} (experiments 2 and 6, Table 1) and confirmed by different *in situ* analysis techniques. The doping concentration of 3% was chosen here due to the non-influence on the calcium phosphate phase transitions in comparison with undoped samples, demonstrated by preliminary *ex situ* XRD analysis (Fig. S6†).

In general, as for the experiments applying Ce^{3+} as a coordination sensor (Fig. 1), no luminescence is initially detected upon the presence of pure $(\text{NH}_4)_2\text{HPO}_4$ solution in the reactor. On addition of the calcium and cerium solution, a broad emission band between *ca.* 310 nm and 440 nm with a maximum at 353 nm constantly rises during the first 10 minutes of the reaction, indicating the formation of the product (Fig. 1), identified as a mixture of ACP and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ by *ex situ* XRD analysis. This emission range is attributed in the literature to

the Ce^{3+} electronic transition from the lowest 5d state to the ground state levels $^2\text{F}_{5/2}$ and $^2\text{F}_{7/2}$ in, for instance, calcium hydroxylapatite (HAp).³⁸ The non-symmetric shape of the Ce^{3+} band can be assigned to the simultaneous presence of secondary phases³⁹ *e.g.* ACP. After $t = 13$ min, the emission intensity starts to decrease and is slightly red shifted, corresponding to the transition to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ indicated by *ex situ* XRD measurements (Fig. S6b†).

The decrease of the emission intensity after $t = 13$ min could be justified by two different explanations. The first possible explanation is the increased quenching effect of the Ln^{3+} luminescence, caused by the two coordinating water molecules on the doped Ca^{2+} site on $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$.^{40,41} Within the second possible explanation, since the Ce^{3+} *in situ* emission spectra are recorded under a constant excitation at 280 nm, the decrease of the emission intensity could be caused by a shift of the excitation spectra during the conversion between different calcium phosphate phases. In order to verify this hypothesis, *in situ* excitation spectra ($\lambda_{\text{em}} = 365$ nm, experiment 3, Table 1) have been recorded under the same experimental conditions as Fig. 1 (Fig. S13–S15†). As shown in Fig. S15,† the time-dependent profile of the *in situ* excitation spectra is very similar to the one of the *in situ* emission spectra and no significant shift in the excitation spectra is observed. Here, the decrease of the intensity of the excitation bands is singly related to the same intensity decrease of the respective emission band at 365 nm. Therefore, this hypothesis can be ruled out and the variation of the time-dependent emission intensity can be associated with the attachment and detachment of quenching water molecules within the coordination spheres of the lanthanide-based coordination sensors. The red shift of the Ce^{3+} emission band upon the formation of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, could indicate that this compound presents a higher coordination number or a shorter average bond length with the coordinating oxygen ions than in the previously formed ACP phase, since it is not the case for $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$.⁴² However, due to the lack of long-range order and Röntgen amorphous character of the ACP phase, very little structural information is available about this phase.^{43,44} In this context, it is important to note that *ex situ* XRD analysis shows the formation of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ after $t = 10$ min (Fig. S6b†), while *in situ* measurements locate this phase transition at $t = 13$ min. As observed in our previous work,⁴⁵ the sample preparation conditions can lead to the *ex situ* conversion of the phase mixture to the stable $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ compound. Within the *in situ* luminescence measurements, the Ce^{3+} emission intensity remains approximately constant at $t = 20$ –40 min, indicating no significant structural change in this time range, in agreement with the respective *ex situ* XRD patterns (Fig. S6b†). After $t = 40$ min, the temperature starts to increase and the emission intensity starts to decrease, caused by thermal quenching effects and confirmed by the consecutive increase of the emission intensity after cooling the system down to room temperature (Fig. 1 and S5†). At approximately, $t = 51$ min, the emission intensity slightly increases and decreases again, indicating additional structural changes in

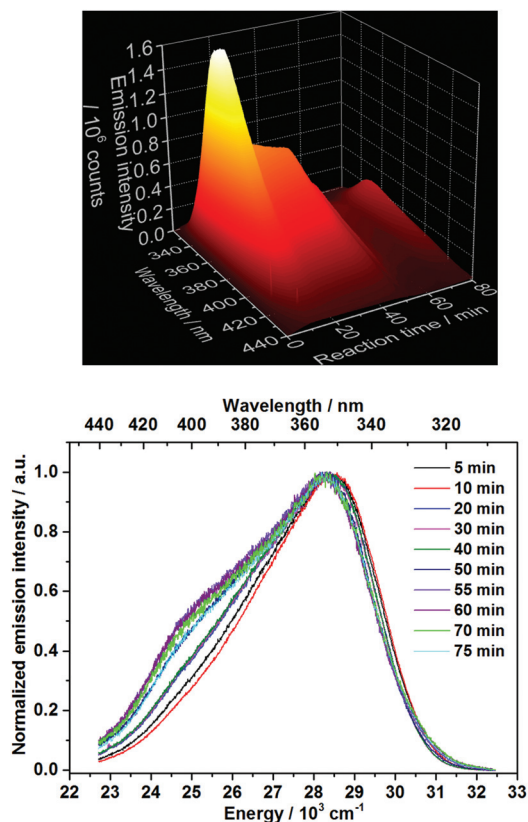


Fig. 1 Top: *In situ* emission spectrum recorded during calcium phosphate phase transitions applying 3% Ce^{3+} as a coordination sensor ($\lambda_{\text{ex}} = 280$ nm). Bottom: Normalized emission spectra for selected reaction times (experiment 2, Table 1).



the cation environment and therefore a new phase transition by reaching the temperature of *ca.* 60 °C. This phase transition coincides with the results obtained by *ex situ* XRD analysis (Fig. S6b†), showing the conversion from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ to $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ between $t = 50$ and 55 min. The temperature of approximately 60 °C also coincides with the decomposition temperature of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ on the *in situ* XRD data measured at the DESY synchrotron facility, discussed in detail below. The formation of the new phase is also indicated by the rise of an additional Ce^{3+} emission band at $24\,500\text{ cm}^{-1}$ (Fig. 1), which is not related to the increase of the temperature, since it is still observed after the system was cooled down to room temperature. The rise of additional Ce^{3+} emission bands is in agreement with the enhancement of crystallographic available Ca^{2+} doping sites for the coordination sensors, increasing from one Ca^{2+} site in $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ to eight Ca^{2+} sites on $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$.⁴¹ The red shift of the Ce^{3+} emission bands can be explained by the decrease of the average bond lengths between the calcium and coordinating oxygen ions within $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$,⁴⁶ caused by the so-called nephelauxetic effect.³⁹ Moreover, additional structural information can explain the spectroscopic behavior, in which the increase of the emission intensity at $t = 51$ min during the phase transition occurs most probably due to the decrease of the number of the quenching water molecules on the coordination sphere of the coordination sensor during the conversion from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ to $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$, further decreasing afterwards due to thermal quenching effects.

Fig. 2 shows the time-dependent emission spectra applying Eu^{3+} as coordination sensors. Since these measurements have been carried out in solution, a strong quenching effect is observed, caused by the non-radiative depopulation of the excited states of Eu^{3+} due to the vibrational energy transfer involving the high energy vibrations of the OH oscillator from the H_2O solvent molecules.⁴⁷ This quenching effect results in the low intensity of the Eu^{3+} emission and consequent

enhanced loss of resolution, differing therefore from previously reported emission spectra of Eu^{3+} doped ACP⁴⁸ and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$.^{49–51} Additional causes for deviations in comparison with luminescence spectra of Eu^{3+} -doped calcium phosphates reported in the literature are different synthesis methods applied and therefore different particle sizes, doping concentrations, measurement temperatures and excitation wavelengths. As mentioned above, no spectral data about the luminescence properties of Eu^{3+} doped $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ is available in the literature so far for comparison. The intensity of the $^5\text{D}_0 \rightarrow ^7\text{F}_j$ ($j = 1–4$) Eu^{3+} transitions shows the same behavior as for the Ce^{3+} experiments. Hence, the continuous increase of the intensity in the first 10 minutes (Fig. 3) indicates the formation of a solid material, assigned to a mixture of ACP and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ by *ex situ* XRD (Fig. S6b†) followed by a decrease of intensity at $t \approx 13$ min, indicating the conversion to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, most probably caused by the quenching effect of the two coordinating water molecules. Also for the Eu^{3+} coordination sensor, the intensity remains constant up to approximately $t = 51$ min, reaching the temperature of 60 °C, when an additional intensity oscillation indicates the additional phase transition to $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$.

Fig. 3 shows in addition the confirmation of the structural changes detected by *in situ* luminescence measurements discussed above by comparison with *in situ* pH value and ion conductivity measurements as well as *in situ* infrared spectroscopy. Up to $t = 10$ min, the conductivity increases during the addition of extra ions during the introduction of the solution containing calcium and cerium or europium nitrate. This solution addition also causes the decrease of the pH, due to the acidity of the nitrate solution. Initially, $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ and ACP ($\text{Ca}_9(\text{PO}_4)_6$)^{11,42} formed, according to the *ex situ* XRD measurements (Fig. S6b†). The higher pH of the initial phosphate solution inside the reactor (pH = 5.85, Fig. 3, orange curve) can cause the deprotonation of the phosphate anions, stabilizing the PO_4^{3-} species and therefore, the $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ and ACP phases.¹¹ Upon the addition of more acidic nitrate solution and consequent decrease of the pH value, the reflec-

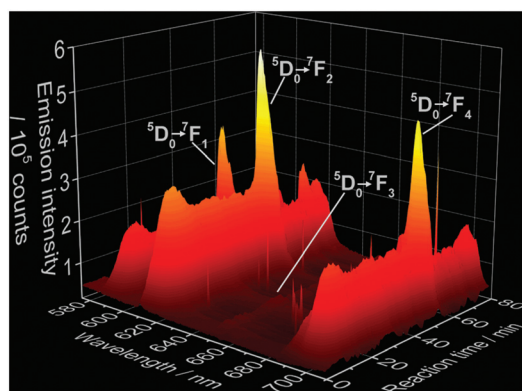


Fig. 2 Time-dependence of $^5\text{D}_0 \rightarrow ^7\text{F}_j$ ($j = 1–4$) electronic transitions of Eu^{3+} during the formation of doped calcium phosphate ($\lambda_{\text{ex}} = 395\text{ nm}$, experiment 5, Table 1). Sharper peaks parallel to the Eu^{3+} emission bands are assigned to measurement artifacts originated by the CCD detector.

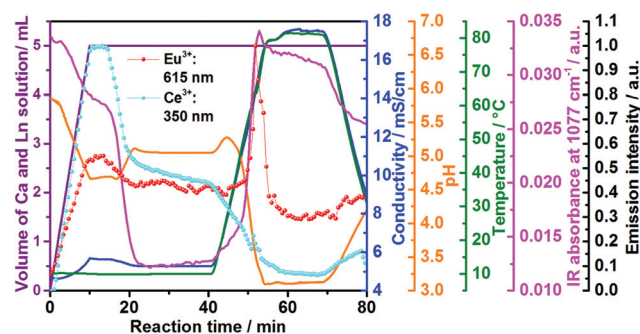
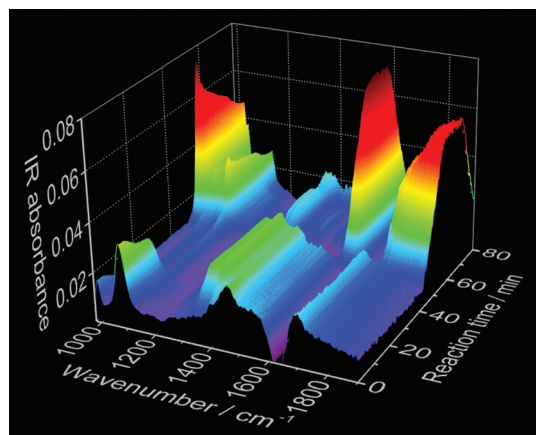


Fig. 3 Time dependence of the emission intensity of Ce^{3+} (light blue curve) and Eu^{3+} (red curve) doped calcium phosphate, *in situ* ion conductivity (dark blue curve), *in situ* pH (orange curve) and IR intensity at 1077 cm^{-1} (pink curve) in comparison with the volume of the Ca^{2+} and Ln^{3+} solution (violet curve) to the reactor containing aqueous $(\text{NH}_4)_2\text{HPO}_4$ (experiments 2 and 6, Table 1).





tions assigned to $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ become more clear and with further decrease of the pH value, the phosphate ions are partially protonated,¹¹ resulting in the conversion from $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$. This conversion also causes a slight decrease in the conductivity due to the uptake of the H^+ ions for $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ formation. Similarly to *in situ* luminescence measurements, *in situ* pH and ion conductivity remain nearly constant between $t = 20\text{--}40$ min, indicating no significant structural changes and the stabilization of the $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ phase (Fig. S6b[†]). After $t = 40$ min, the large oscillations on pH values and ion conductivity are strongly influenced by the temperature, masking additional structural changes. Also interesting are the changes in the IR absorption bands during the calcium phosphate phase transitions (Fig. 3 and 4), displayed in detail in the ESI (Fig. S16[†]). Fig. 3, for instance, shows the time-dependence of the IR absorption band at 1077 cm^{-1} , assigned to the δPOH vibrations within the $(\text{NH}_4)_2\text{HPO}_4$ solution.⁵² Initially, the intensity of this band decreases due to the uptake of phosphate ions from the solution to form ACP and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, after deprotonation. The decrease of this IR band is also caused by the uptake of HPO_4^{2-} ions for the formation of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ($\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$).

As mentioned above, since samples analyzed *ex situ* can be influenced by the preparation procedure, *in situ* XRD measurements at the P02.1⁵³ and P08⁵⁴ beamlines at DESY in Hamburg, Germany, have been additionally carried out (Fig. S17–S21†). In general for all *in situ* experiments, an increase of the background at low 2θ angles is observed upon the addition of the solution containing calcium and europium or cerium nitrate to the $(\text{NH}_4)_2\text{HPO}_4$ solution. This background increase is assigned to the formation of an amorphous phase,⁵⁵ coinciding with the formation of the combined ACP and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ phases demonstrated by the *ex situ* XRD

According to Engelke *et al.*,⁵ different mechanisms can govern the transformation between different phases during the formation of solid materials in solution. These mechanisms are, for instance, (i) a direct solid–solid transition, (ii) the first phase completely dissolves before the emerging phase nucleates and crystallizes from the solution and (iii) the formation of the single phase consists of completely separated processes. Fig. S18† shows, for example, that the intensity of the ACP XRD signal is highly correlated with the intensity of the (0,2,0) reflection of the $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ phase. Hence, the onset of the decay of the ACP occurs simultaneously with the onset of the crystallization of the $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ phase, indicating that $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ grows and the intensity of these reflections increases at the cost of the ACP phase. Therefore, the hypothesis (iii) can be ruled out. If hypothesis (ii) were true, the ACP signal would partially or completely disappear before the onset of the $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ crystallization and the curves of the normalized reflection intensities of these two phases would not intersect. A similar behaviour can be observed comparing the correlation of the intensities of the reflections assigned to the $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ phases. The normalized reflection intensities of the respective transitions show, however, intersections at 0.5 and 0.6 (Fig. S18†), indicating that the respective conversions from ACP to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ to $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ are rather governed by solid–solid phase transitions, in agreement with hypothesis (i).⁵⁶

Fig. S18† shows the normalized intensity of XRD intensities ($\lambda = 0.4959 \text{ \AA}$) at 0.5732° 2θ (green curve), assigned to the amorphous phase,⁵⁵ 1.5142° 2θ (pink curve), assigned to the (1,0,0) reflection of $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ⁴⁶ (Fig. S17†) and 3.7471° 2θ (violet curve), assigned to the (0,2,0) reflection of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ⁴¹ (Fig. S19†), measured *in situ* during the synthesis of Eu^{3+} -doped calcium phosphate at the DESY P08 beamline (experiment 9, Table 1). This diagram shows the initial formation of amorphous calcium phosphate, which converts at $t = 7.5\text{--}13.5 \text{ min}$ to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, growing further up to $t = 22 \text{ min}$. When the temperature is increased to 63°C , the intensity of the reflection assigned to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ starts to decrease, upon the increase of the reflection assigned to the $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ phase. In this context, it is important to note that the increase of the intensity of the reflection at

1.5142° 2 θ (pink curve) at $t = 0\text{--}14$ min occurs due to the overlap with the broad signal of the amorphous phase and not because of a premature formation of $\text{Ca}_3\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$. As explained within section 2.2, slight shifts in the time range of the phase transitions in comparison with experiments carried out with Setup A might be assigned to the adaptation of the experimental conditions, necessary for performing these experiments at the synchrotron facility.

The formation of the amorphous phase at $t = 1$ min is also detected by the simultaneous increase of the intensity of the $\text{Eu}^{3+} {}^5\text{D}_0 \rightarrow {}^7\text{F}_2$ electronic transition at 613 nm. The changes in the intensity ratio between the ${}^5\text{D}_0 \rightarrow {}^7\text{F}_2$ and ${}^5\text{D}_0 \rightarrow {}^7\text{F}_1$ transitions indicate changes in the symmetry around the cation sites during the formation of the amorphous phase (Fig. S19[†]).

Similar to Fig. S18,† Fig. 5 shows the initial formation of ACP during the synthesis of Ce^{3+} -doped calcium phosphate, which converts at $t = 9\text{--}14.5$ min to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (Fig. S21†), growing further up to approximately $t = 23$ min. Similar to that indicated in Fig. S6,† a possible explanation for the longer stabilization of the amorphous phase on the measurements in Fig. 5 (7% Ln^{3+}) than the measurements in Fig. S18† (5% Ln^{3+}) could be the higher amount of coordination sensors. Because the differences in ionic radii and charge between Ln^{3+} and Ca^{2+} ions, doping trivalent lanthanides within calcium phosphate could increase the disorder within the solid material, delaying crystallization. When the temperature is increased to *ca.* 60 °C, the intensity of the reflection assigned to $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ decreases, upon the increase of the reflection assigned to the $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ phase (Fig. S20†). As previously mentioned in Fig. S18,† the increase of the intensity of the reflection at $0.6347^\circ 2\theta$ (pink curve) at $t = 0\text{--}20$ min occurs due to an overlap with the broad signal of the amorphous phase and not because of a premature formation of $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ (Fig. S21†). The intensity of the reflection

assigned to $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ reaches its maximum at the maximum temperature of 100 °C and decreases afterward turning the heating system off at $t = 28.5$ min, which could be caused by (i) a decrease of crystallinity or (ii) the partial dissolution of the product. Hence, the simultaneously performed *in situ* measurements of light transmission through the reaction solution offer additional insights into this open question and is discussed in detail in Fig. 6. At $t = 55$ min, a 25% NH_3 solution was added to the reactor in order to evaluate the effect of the increase of the pH in this reaction stage. However, the addition of NH_3 has not shown a significant influence on the calcium phosphate phase transitions.

As explained in section 1.2 of the ESI,[†] for these experiments, a 365 nm light source was used for irradiating the reactor, while an optical fiber submersed in the reactor content and connected to a CCD detector, was applied for measuring the intensity of the light source transmitted through the solution during the reaction. Upon the addition of the calcium and cerium nitrate solution to the reactor containing $(\text{NH}_4)_2\text{HPO}_4$ (Fig. 6), the intensity of the light source firstly increases, probably due to a brief diluting effect caused by the addition of the second solution to the reactor. The formation of solid material at $t = 0.5$ min is detected by the strong decrease of the intensity of the light source, caused by the increase of the turbidity of the solution, blocking light transmission.⁴⁵ The light transmission decreases further up to $t \approx 14$ min, during the formation of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (Fig. 5), and is rather constant while the growth of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ stabilizes. At approximately $t = 23$ min, the light transmission starts to decrease again, coinciding with the formation of $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ (Fig. 5). Even though the intensity of the reflections decreases during the cooling process, the further decrease of the light intensity indicates an increase in the turbidity of the solution, ruling out the hypothesis of product dissolution, discussed in Fig. 5. The oscillation of the intensity of the light source and signals of all other sensors at $t = 28.5$ min are assigned to the oscillation of the temperature by turning off the heating system.

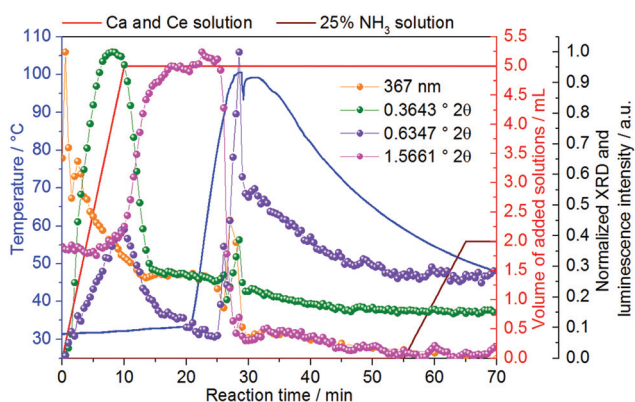


Fig. 5 Normalized intensity of XRD reflections ($\lambda = 0.2075 \text{ \AA}$) at $0.3643^\circ 2\theta$ (green curve), assigned to the amorphous phase,⁵⁵ $0.6347^\circ 2\theta$ (pink curve), assigned to the (1,0,0) reflection of $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ⁴⁶ (Fig. S20†) and $1.5661^\circ 2\theta$ (violet curve), assigned to the (0,2,0) reflection of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ⁴¹ (Fig. S20†), measured *in situ* during the formation of Ce^{3+} -doped calcium phosphate at the DESY P02.1 beamline (experiment 10, Table 1).

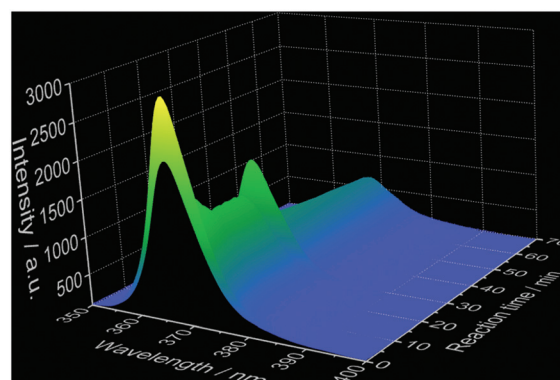


Fig. 6 Time-dependent light transmission of a 365 nm light source during the synthesis of Ce^{3+} -doped calcium phosphate (experiment 10, Table 1).

4. Conclusions

This work introduces the recently developed ILACS²⁶ *in situ* luminescence approach as a new technique for characterizing the phase transitions in calcium phosphate. Calcium phosphate is a major inorganic component of the human body,¹¹ often found in both healthy and pathologic tissues such as bones and teeth, heart calcifications or caries. Here, the ILACS approach explored the coordination sensitive emissive f-d and f-f electronic transitions on, respectively, Ce³⁺ and Eu³⁺ as local sensors for delivering information about structural changes around the doped cation sites. Therewith, it was possible to detect the formation of ACP and Ca₅(PO₄)₃OH as well as their subsequent conversion to CaHPO₄·2H₂O and Ca₈H₂(PO₄)₆·5H₂O. The detection of these phase transitions has been confirmed by additional characterization methods as *in situ* measurements of pH value, ion conductivity, IR spectroscopy, besides *ex situ* and synchrotron-based *in situ* techniques. Upon addition of the solutions of calcium and europium or cerium nitrates to (NH₄)₂HPO₄, a mixture of ACP and Ca₅(PO₄)₃OH is formed, converted to CaHPO₄·2H₂O, most probably due to a decrease of the pH. On increasing the temperature, CaHPO₄·2H₂O is decomposed upon the formation of CaHPO₄ with a higher reactant concentration and Ca₈H₂(PO₄)₆·5H₂O with a lower reactant concentration. Understanding the phase transitions of calcium phosphate is important for, for instance, comprehending and treating diseases.

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