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Real-Time Raman Monitoring of Calcium Phosphate Precipitation in a Semi-Batch Stirred Crystallizer

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ABSTRACT: Semi-batch precipitation of calcium monohydrogen phosphate dihydrate (CaHPO₄·2H₂O; also termed dicalcium phosphate dihydrate, DCPD) from a homogeneous reaction of KH₂PO₄ and CaCl₂ was studied with inline Raman spectroscopy. In the reaction, hydroxyapatite (HAP, Ca₅OH·(PO₄)₃) formed first and transferred to DCPD throughout the entire precipitation process. Raman calibration models were created with a series of KH₂PO₄–NaOH–water solutions. The reliability of the calibration models was validated with offline measurement of phosphate concentration in the mother liquor. Real-time changes in HPO₄²⁻ and H₂PO₄⁻ concentration were monitored and predicted. Comprehensive kinetic information was obtained from liquid and solid analysis. It was found that the influence of the mixing rate on the precipitation was not significant, whereas the reactant feeding rate had a remarkable impact.

1. INTRODUCTION

In recent years, recovery of phosphorus has become a subject of great research interest. This interest stems not only from phosphorus being an important, geographically concentrated, nonrenewable resource required to support food production but also because easily accessible natural reserves, such as phosphate rocks, are becoming depleted. Moreover, and another consideration is the contamination of natural waters with excess nutrients detrimental to human life and the natural environment. High levels of phosphorus are the cause of eutrophication in rivers, lakes, and seas the world over. Various techniques have been proposed for phosphate removal, including approaches based on biological nutrient removal and physicochemical treatment methods. Recovery and reuse of phosphorus from wastewater, waste streams, and phosphorus minerals is an effective way to reduce risks to agriculture and water quality and thus protect the global environment.

Precipitation of calcium phosphate is an attractive option due to its wide applications. For example, calcium phosphate salts can be used in inorganic bone tissue and other biomedical materials. The precipitation process is, however, rather complex, as different sparingly soluble crystalline phases such as calcium monohydrogen phosphate dihydrate (DCPD, brushite), octacalcium phosphate (OCP, Ca₈H₂(PO₄)₆·SH₂O), and calcium hydroxyapatite (HAP) can be formed during the reaction. The formation of these phosphates depends on operational conditions such as the concentration of reactants, pH, supersaturation level, and temperature. For instance, DCPD with well-defined morphology and adjustable crystallite size can be fabricated with the addition of glutamic acid and arginine. The addition of fluorescein and acetone can have impacts on the size of the DCPD crystals and densities of the stacked layers. Lagno et al. investigated homogeneous and seed-assisted precipitation of DCPD and HAP in supersaturation controlled regimes. Different-sized plate-like crystals of DCPD and nanosized HAP were produced. Crystals with desired particle size and morphology can be obtained at optimal operating conditions, but tools capable of precise control of the process are required. Raman spectroscopy, a popular and relatively uncomplicated inline technology, can feasibly be used to monitor the changes taking place in solid–liquid suspensions during the crystallization process. In a previous study, anti-solvent crystallization of KH₂PO₄ in binary solvents of acetone and water systems were successfully monitored by Raman spectroscopy. The real-time concentration profiles of the solute and solvent could be captured and quantitatively analyzed, which gave comprehensive information about the supersaturation profiles. At the same time, solid information could also be recorded by Raman spectroscopy, which provided information about real-time changes during the crystallization process. Moreover, polymorphic forms can also be detected by inline Raman monitoring. Raman spectroscopy is thus a powerful tool providing information about real-time changes in both liquid phase and the solid state.

General forms of phosphate anions, including HPO₄²⁻, H₂PO₄⁻, and PO₄³⁻ in solution, and numerous calcium and...
phosphate phases may be present in reactive crystallization of calcium phosphates, which poses challenges to understanding and assessment of the dynamic behavior of the process. To our best knowledge, application of Raman spectroscopy to real-time monitoring of calcium phosphate precipitation has not been investigated widely.

The present work focuses on precipitation of calcium phosphate from the reaction between CaCl₂ and KH₂PO₄ at ambient temperature and pressure. In the study, the suitability of inline Raman spectroscopy for monitoring of the precipitation process is evaluated, and it is found that Raman spectroscopy can be successfully used to observe real changes in both the mother liquor and the solid phase, which is the main finding of the present work. In addition, the work investigates the effects of the stirring speed and feeding rate. The results of real-time monitoring provide systematic information about the complex reactive crystallization and contribute to better understanding of the fundamental mechanism and kinetics of the calcium monohydrogen phosphate dihydrate precipitation process, thus enabling control and optimization of the process for future research work.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods. Analytical grade KH₂PO₄ (purity ≥ 99.5%, Merck) and CaCl₂·2H₂O (purity ≥ 99.0%, VWR) were used without further purification. Deionized water was used to prepare the reactant solutions.

In each semi-batch operation, 1 L of KH₂PO₄ solution (0.2 mol·L⁻¹) was placed in a 2 L glass reactor. Four baffles were located symmetrically in the reactor to promote efficient mixing and prevent vortex formation. The inner diameter of the crystallizer was 150 mm and its height was 230 mm. A 50 mm Rushton turbine with six blades was used as a stirrer. The pH of the KH₂PO₄ solution was adjusted to 8.5 with 5 M NaOH at the beginning of the experiments. 1 L of CaCl₂·2H₂O solution (0.2 mol·L⁻¹) was prepared and pumped into the reactor with a metering and transfer pump (Watson Marlow 323) at different flow rates (10, 20, and 50 mL/min). The inlet of the feeding tube was located at the same level as the impeller and pointed directly toward the center of the reactor. A multiparameter analyzer (consisting of a pH meter, a conductivity meter, and a temperature meter) connected to a PC was used for inline monitoring of changes in the suspension. An inline Raman probe was immersed into the crystallizer to monitor the process. After addition of CaCl₂ solution, the suspension was stirred for 1 h to ensure a steady state. During the precipitation process, slurry samples were taken at different times to investigate the crystallization kinetics. After filtration with a Büchner funnel, the liquid sample was analyzed by ion chromatography (ICS-1100 from Thermo Scientific Inc.) to determine the total concentration of phosphates. The filtered crystals were dried at room temperature for further analysis. The mixing rate was also considered as a variable. Figure 1 shows a schematic diagram of the experimental setup. Particle size distributions were determined by a Malvern Master sizer 3000 laser particle size analyzer. The composition and morphology of the crystals were examined by X-ray powder diffraction (XRD, Bruker D8) and scanning electron microscopy (SEM, Hitachi SU3500), respectively.

2.2. Raman Monitoring. 2.2.1. Raman Spectroscopy. Raman spectra were collected with a Kaiser Raman RXN11 spectrometer at 785 nm laser excitation with a maximum power of 400 mW. A BHO-LAB probe with a flexible MR probe head and bio immersion optic was used for in situ measurement. This probe features a fixed-focus approach that not only provides long-term measurement stability but also delivers a high signal-to-noise ratio at any length of optical fiber. The spectra were captured with an exposure time of 45 s with a sampling interval of 120 s for the feeding rate of 10 mL/min, and an exposure time of 30 s with a sampling interval of 70 s for the feeding rate of 50 and 20 mL/min with two accumulations. In order to ensure that enough spectra can be taken and strong Raman signals can be detected, the two exposure times were used with all three studied semi-batch residence times of 20, 50, and 100 min. All the Raman spectra were treated in the same way by icRaman software in order to avoid any differences in data treatment. Baseline correction of the third order was performed prior to normalization.

2.2.2. Raman Calibration. For quantitative analysis of solute concentration with Raman spectroscopy, a calibration model is always needed to correlate the Raman spectrum with the solute concentration in the mother liquor. Generally, the calibration should be done in the range where the actual measurement takes place. However, this is not possible for crystallization processes since crystallization always happens in a supersaturated solution, which may cause large errors due to the metastable state. Furthermore, a dense crystall phase may exert an effect on the spectra which has to be considered in the calibration. However, the crystal effect on the spectra can be eliminated by normalizing the characteristic peak height of the solute with characteristic peak height of the solvent.11,12 In the Raman calibration and prediction of the current work, the same effect of crystals on the characteristic peak height of the HPO₄²⁻ and H₂PO₄⁻ in the solution was assumed. All the spectra were treated in the same way by the third-order baseline correction and normalization with the water band at 1643 cm⁻¹ in order to minimize the effect of solids on the Raman spectra.

Since the solution pH is shifting throughout the precipitation process, six solutions with different pH levels in the range of 4.3–8.5 were used to generate the Raman calibration model. First, a KH₂PO₄ solution with a concentration of 0.2 mol·L⁻¹ was prepared (pH 4.3). Then, with the addition of 10, 20, 30, 40, and 41 mL of NaOH (5 M), five solutions of different pH were prepared. The solutions had a pH value of 6.2, 6.7, 7.1, 8.1, and 8.5, respectively. Raman spectra were taken with the exposure time of 30 and 45 s with two accumulations for each calibration solution. During the pH adjustment process, the chemical reaction shown in eq 1 takes place. The concentration of H₂PO₄⁻ decreases, and HPO₄²⁻ concentration increases due to the presence of NaOH in the clear solution. These changes were observed by Raman spectroscopy, and the changes can be clearly seen in Figure 2.

\[
\text{H}_2\text{PO}_4^- + \text{OH}^- \leftrightarrow \text{HPO}_4^{2-} + \text{H}_2\text{O} \tag{1}
\]

From Figure 2, it can be seen that the Raman characteristic peaks at 875 and 1077 cm⁻¹ reduced, and the peak at 990 cm⁻¹ increased with the addition of NaOH. Moreover, the shape of the Raman peak at 875 cm⁻¹ changed, which was seen particularly clearly when 41 mL of NaOH solution was added. The same phenomenon and trend was observed for the calibration results with exposure time of 45 s and 30 s. Consequently, Raman peaks at 990 and 1077 cm⁻¹ were selected and used to represent the concentration of HPO₄²⁻ and H₂PO₄⁻, respectively. The crystals of DCPD and HAP are located at the Raman shift of 986 and 957 cm⁻¹, respectively. This can be seen in Figure 5 and is supported by XRD examination. These results are similar to the reference data.53 It can be observed that the DCPD characteristic peak at 986 cm⁻¹ overlapped with the peak at 990 cm⁻¹.

![Figure 1. Schematic diagram of the experimental setup.](image)
which represents the HPO$_4^{2-}$ anion. This overlap requires special attention when determining the Raman calibration model. Therefore, in this case, the height of the peak at 990 cm$^{-1}$ and the area of the peak at 1077 cm$^{-1}$ were chosen for construction of the univariate calibration models. The calibration curves and models shown in Figure 3 for the HPO$_4^{2-}$ and H$_2$PO$_4^{-}$ ions were then generated to predict the concentration of the two ions in the mother liquor. It can be seen from the high values of the determination coefficient $R^2$ that the calibration models are reasonably accurate. Predictions were always done with the calibration model that had the same exposure time and sampling interval.

3. RESULTS AND DISCUSSION

3.1. Effects of Feeding Rate and Mixing Rate on Precipitation. Three feeding rates of reactant CaCl$_2$ and two mixing rates were considered in the present work. In the semi-batch crystallization experiments, the obtained results clearly indicated that the formation of calcium phosphate is remarkably sensitive to pH. According to the temperature recording, the temperature change was within 0.9 °C, which means that temperature remained almost constant throughout the reactive crystallization. Figure 4 shows trends for pH and conductivity as a function of time for the semi-batch precipitation processes. It can be seen from Figure 4a that the pH continuously reduces until all the CaCl$_2$ solution has been added to the KH$_2$PO$_4$ solution, after which the pH then increases, before stabilizing at the end of the measured period. There is no significant difference in pH with different mixing rates during the reaction. The pH reaches the constant level faster with a stirring rate of 500 rpm, which suggests that the system reaches equilibrium faster with a higher mixing rate. The final pH is the same for all the precipitation processes. However, the lowest pH with a pumping time of 100 min was slightly higher than that of the other pumping times. The trends of conductivity with the longest pumping time are quite different from the other pumping times, as seen in Figure 4b. The conductivity trends are very similar to the pH trends, but there is a reduction period immediately after the addition of the reactant solution for the pumping time of 50 and 20 min.

From another point of view, as shown in Figure 4c, it was noticed that the pH started to differ with different experimental conditions when 0.7 L of CaCl$_2$ solution was added. At this point (i.e., 14, 35, and 70 min with a feeding rate of 50, 20, and 10 mL/min, i.e., pumping times 20, 50, and 100 min, respectively), pH reached around 6 and the formation of DCPD started. These results are very similar to the findings reported by Liubov et al., who stated that the mixtures of DCPD and HAP were obtained in the pH range of 5.7–6.3. Moreover, from Figure 4d, it can be seen that the turning points of conductivity with various conditions also occurred while adding 0.7 L of CaCl$_2$ solution. It also indicates that DCPD started to precipitate, which is also supported by the observation of XRD and Raman for solid analysis. With a lower feeding rate of 10 mL/min, uniform and flocculent HAP crystals can be obtained as clearly seen from Figure 8b, whereas
nonuniform HAP particles consisting of small spherical crystals were precipitated from the other two feeding rates.

Figure 5 shows selected Raman spectra from semi-batch precipitation of calcium phosphate at a pumping time of 100 min and mixing rate of 350 rpm at room temperature. It can be clearly seen from Figure 5 that the peak at 990 cm$^{-1}$ first reduces and then disappears around 70 min, whereas the peak at 1077 cm$^{-1}$ rises continuously and reaches a maximum around 70 min. Changes in the peak show the decrease of the HPO$_4^{2-}$ concentration and increase of the H$_2$PO$_4^-$ concentration. After that, a peak at 986 cm$^{-1}$ appears and increases significantly until the end of the process, which indicates that solid DCPD precipitated from the reaction. Simultaneously, it can be noticed that the peak at 958 cm$^{-1}$ increases gradually from the beginning of the reaction, then decreases after 70 min, and almost disappears at the end. This can be attributed to the formation of HAP in the process. Solid analysis results obtained from Raman spectroscopy for the batch with a mixing rate of 350 rpm and pumping time of 100 min shown in Figure 6 illustrate the composition of the precipitated crystals, which is consistent with XRD results. XRD patterns of the samples obtained before 60 min agree well with reference data JCPDS 09-0432, which indicates the formation of HAP. Three new characteristic reflections at 11.7º, 21.0º, and 29.3º (2θ) that were observed from 60 min were consistent with the reference data JCPDS 09-0077, which means DCPD started to precipitate. From 70 min, the main solid compound in the slurry is DCPD, whereas HAP is reacted gradually. The same phenomenon can be seen from the results of Raman spectroscopy shown in Figure 6. The Raman results are consistent with the reference data.$^{13}$

Two typical measured concentration profiles are shown in Figure 7. The tendencies of concentrations of H$_2$PO$_4^-$ and HPO$_4^{2-}$ ions in the other obtained profiles are similar to those in Figure 7. During the precipitation process, the concentration of HPO$_4^{2-}$ constantly reduced, whereas the concentration of H$_2$PO$_4^-$ increased to a maximum and then decreased to close...
to zero at the end of the measurement period. The sum of the concentration of $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ ions from the inline analysis (white triangles $\Delta$ in Figure 7) can be compared with the offline analysis (black triangles $\blacktriangle$ in Figure 7) that shows the total amount of phosphate in each sample. The good agreement between the inline and offline measurements demonstrate the accuracy of the Raman analysis and the solid spectra of HAP and DCPD do not have an impact on the spectral analysis of the ions in the mother liquor. The inline Raman spectroscopy allows monitoring of changes in individual substances that cannot be determined or are difficult to determine by offline analysis. Comparison of the inline and offline analysis results showed that the developed calibration models were reliable and accurate.

Similar trends of solids analysis and mother liquid analysis were also observed from other semi-batch processes. For instance, with both studied mixing rates, DCPD started to precipitate, observed by Raman and XRD analysis, from 40 and 15 min for the pumping time of 50 and 20 min, respectively. Thus, the mixing rate had no significant influence on the precipitation, which is a finding that corresponds to the results of pH and conductivity shown in Figure 4. The morphology of the HAP crystals precipitated, shown in Figure 8a, is small, porous, and roughly spherical, which is very similar to the results reported in ref 8.

The reactant feeding rate, however, has a major effect on the precipitation. When a pumping time of 100 min was used, there was almost no HAP precipitate present at the time of completion of addition of CaCl$_2$, i.e., 100 min. DCPD with plate-like features shown in Figure 8c was mainly precipitated. The crystal morphology of DCPD is similar to the result reported in ref 8. With the other two pumping rates, quite a few HAP crystals were still present in the solution, which suggests that HAP was not completely transferred to DCPD. Moreover, there was a higher amount of HAP when the addition of CaCl$_2$ was completed in a pumping time of 20 min (Figure 8h) than a pumping time of 50 min (Figure 8f). This can be explained by HAP formation dominating the process, since a higher reaction rate and precipitation rate was reached with a higher feeding rate. Two results support this conclusion: (i) The lowest pH value reached with a pumping time of 20 and 50 min was a bit higher than that with a pumping time of 100 min, and (ii) the concentration of phosphate in the mother liquor was lower with the longest pumping time. Therefore, it would appear that priority precipitation of HAP reduces the nucleation rate of DCPD. Consequently, a large amount of HAP is still present at the end point of CaCl$_2$ addition. Thus, after addition of the reactant, the transformation from HAP to DCPD continues. Panels (b), (e), and (g) in Figure 8 show the morphology of precipitates when DCPD starts to form around 60, 40, and 15 min for a pumping time of 100, 50, and 20 min, respectively. The end product of all the investigated semi-batch precipitations of calcium phosphate was DCPD with a plate-like crystal morphology, an example of which is shown in Figure 8d. From the conductivity results in Figure 4b, it can be seen that there is an increase in conductivity at almost the same times of 15, 40, and 60 min for pumping times of 20, 50, and 100 min, respectively, as the onset of formation of DCPD detected by Raman spectroscopy. However, this needs further study due to the complexity of the conductivity of supersaturated solutions.
The conductivity only estimates the total ion concentration and depends on many factors such as the specific nature of the ions, the concentration of the ions, the temperature, and the crystal properties.

Particle size distribution of the end products from each semi-batch process is shown in Figure 9. A minor increase in particle size of DCPD with a decrease of pumping time was observed for both mixing rates.

### 3.2. Mechanism of Precipitation

On the basis of the results obtained from liquid and solid phase analysis, the following mechanism for the studied semi-batch precipitation process of calcium monohydrogen phosphate dihydrate is proposed. As the starting pH of the solution was 8.5, divalent phosphate ion, HPO$_4^{2-}$, is mainly present in the solution and almost no H$_2$PO$_4^-$ exists at the beginning. With the addition of CaCl$_2$ solution, HAP is first formed by the reaction shown in eq 2. Therefore, the concentration of HPO$_4^{2-}$ reduced and it also explains the decrease in pH. This result is similar to the findings reported by Recillas et al., who found that the first precipitates formed were HAP when pH was higher than 6 for low concentration of calcium and high concentration of phosphate.

At the same time, since the concentration of HPO$_4^{2-}$ ion is considerably high in the solution, the reaction described in eq 3 occurs. This results in an increase in H$_2$PO$_4^-$ concentration. When 0.7 L of CaCl$_2$ was pumped into the system, it can be seen that the pH of the solution reached around 6, which is the favorite pH to form DCPD as shown in Figure 4c. Thus, H$_2$PO$_4^-$ ion started to react with the OH$^-$ provided by the solid of HAP to produce DCPD. Then, DCPD precipitated due to the reaction shown in eq 4. This phenomenon was observed from the decrease of H$_2$PO$_4^-$ and the solid information on HAP and DCPD. Therefore, DCPD continuously increases, whereas HAP decreases gradually and is reacted completely at the end. These observations agree well with the XRD and Raman results of solid samples.

\[
\begin{align*}
5\text{Ca}^{2+} + 3\text{HPO}_4^{2-} + \text{OH}^- & \leftrightarrow \text{Ca}_3(\text{PO}_4)_2(\text{OH}) \text{ (HAP)} + 3\text{H}^+ \quad (2) \\
\text{HPO}_4^{2-} + \text{H}^+ & \leftrightarrow \text{H}_2\text{PO}_4^- \quad (3) \\
\text{Ca}^{2+} + \text{HPO}_4^{2-} & \leftrightarrow \text{CaHPO}_4\text{2H}_2\text{O} \text{ (DCPD)} \quad (4)
\end{align*}
\]

### 4. CONCLUSIONS

In the present work, precipitation of calcium phosphate was successfully monitored by inline Raman spectroscopy. The developed univariable calibration model was applied to correlate the Raman spectra and the concentration of individual ions in the mother liquor. Good agreement between inline and offline data demonstrated the accuracy and reliability of the Raman analysis. Raman analysis can be used to obtain the concentration profiles of HPO$_4^{2-}$ and H$_2$PO$_4^-$ ions. Moreover, solid analysis by Raman spectroscopy can qualitatively determine the composition of the compounds. Together with other analytical methods, XRD and SEM, reaction kinetics was obtained and a mechanism for the precipitation and transformation of HAP to DCPD was proposed. The pH has a significant effect on the studied precipitation process, since it correlates with the dissociation of various phosphate ions. The initial period exhibits mainly the formation of HAP, after which the precipitation of DCPD starts. The mixing rate was found to have no significant impact on the precipitation. However, the feeding rate of the reactant solution of CaCl$_2$ has a key role in the precipitation as it impacts the supersaturation degree. Particle size increased with increase in feeding rate. This may be explained by the crystal aggregation. Higher feeding rate results in higher supersaturation degree, which leads to higher precipitation rate and the aggregation promotion. It is worthy of notice that the reaction or transformation from HAP to DCPD was not completed when the addition of the reactant solution, CaCl$_2$ solution, ended, especially with shorter pumping time (20 and 50 min). Furthermore, it was seen that the shorter the pumping time, the greater amount of HAP was present in the initial period. Thus, an aging period is needed to produce a pure final product. The concentration results of the mother liquor showed that more than 97% phosphorus had been recovered as DCPD. SEM images indicated that the morphology of the HAP was porous and spherical, whereas DCPD crystals were plate-like in shape.

The methodology developed in the current research work can be applied to other complex aqueous precipitation systems of electrolytes. The Raman analysis method can be used for detection and quantification of dissociated anions, and it has wider potential for use as an accurate and appropriate real-time process monitoring method in precipitation study. Moreover, inline Raman analysis can also be employed in process control and design to obtain desired crystals with certain properties.

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**REFERENCES**


