

# Conductometric Study of Precursor Compound Formation during Wet-Chemical Synthesis of Nanocrystalline Hydroxyapatite

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Conductometry was employed to study the phase evolution of calcium phosphate compounds during the wet-chemical synthesis of hydroxyapatite (HA). Calcium hydroxide and orthophosphoric acid were used to prepare HA at various temperatures ranging from 30 to 95 °C. The electrical conductivity and pH of the reaction mixture were measured at regular intervals of time during acid addition, and the rate of change of conductivity was used to decipher the end point of the reaction. Our previous studies have shown that the end product of this reaction route yields mildly carbonated crystalline HA. The trend of the change in conductivity with time was similar at all temperatures. The conductivity curves were divided into three regions based on the variation in slope of the curves. The slope of the curves decreased with increasing temperatures in the first two regions, and the slope is greater in the second region than in the first. From the conductivity and pH measurement results, the possible precursor phase was identified and it had the composition  $\text{Ca}_3(\text{PO}_4)_2$ . The kinetics of phase transformation was also analyzed and compared to previous work. The similarities between this work and the traditionally adapted experimental work for phase formation and transformation kinetics are highlighted, and the novelty in the current work is discussed.

## Introduction

Precipitation of hydroxyapatite (HA,  $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ) in aqueous media has been studied extensively because of its close resemblance in composition and structure to the mineral part of natural bone, dentin, and enamel.<sup>1,2</sup> There is also impetus to study this reaction due to its implications and applications in industrial wastewater treatment and fertilizer production.<sup>3,4</sup> Crystalline HA is the end product of the reaction between aqueous solutions of calcium salts and dibasic phosphate salts in neutral or basic media. Several other calcium phosphates have been identified as those that form precursor compounds depending on the pH of the solution, from which crystalline HA forms through phase transformations. In slightly acidic solutions the transformation occurs via octacalcium phosphate (OCP,  $\text{Ca}_8\text{H}(\text{PO}_4)_6 \cdot 2.5\text{H}_2\text{O}$ ) and/or dicalcium phosphate dihydrate (DCPD,  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), and in basic solutions (pH > 7.4) it occurs via amorphous calcium phosphate (ACP) with the molecular formula  $\text{Ca}_3(\text{PO}_4)_2 \cdot x\text{H}_2\text{O}$ .<sup>5–10</sup> The precursor compounds formation and their transformation to crystalline HA and transformation kinetics have been studied in systems that closely mimic physiological conditions, that is, studied in a known low or moderately supersaturated solution prepared by mixing the calcium and dibasic phosphate salts.

In laboratory or batch-process production of HA, the phosphate solutions are added to the solution containing calcium

ions in a controlled manner and at a slow rate. The reaction between calcium hydroxide and orthophosphoric acid, used to prepare HA,<sup>11,12</sup> is one such batch process due to its commercial viability. However, the specific kinetics of formation of HA in these batch processes has not been studied in detail. In batch processing, the degree of supersaturation ( $S_{\text{Cap}}$ ) with respect to the precipitating compound continually changes, and hence the stability of the precursor compound and the transformation sequence may differ from the solutions with fixed initial  $S_{\text{Cap}}$ . Also, analyzing the composition of the precursor compound at various stages during the reaction is challenging due to the practical difficulties involved in separating the compound from the solution as well as from other compounds. Since the phase transformation leads to a change in composition in both the solution and the precipitate, in situ measurements on the variation of ionic concentration in solution during the reaction may give an insight into the reaction mechanism. Electrical conductivity measurements of solutions can be considered to be an indirect way of measuring the ionic concentration, and any phase transformation involving compositional changes can be read from the variation of conductivity with time. In the present study, such an attempt is made to study precursor compound formation by measuring the conductivity of the reaction mixture, during batch-process synthesis of HA.

## Materials and Methods

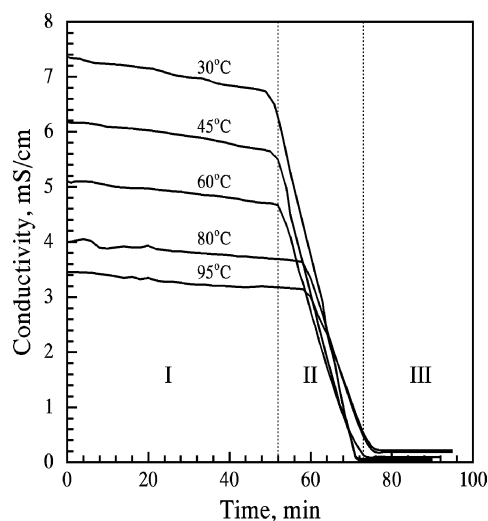
The formation of HA from the reaction between  $\text{Ca}(\text{OH})_2$  and  $\text{H}_3\text{PO}_4$  is described in Scheme 1. The chemicals used in this study were reagent grade and were dissolved in deionized

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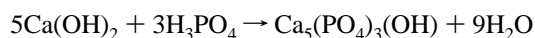
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**Figure 1.** Variation of conductivity with reaction time at different temperatures.

(DI) water produced using a Milli-Q unit (Millipore, USA). The conductivity of the DI water was  $0.05 \mu\text{S}/\text{cm}$  at  $25^\circ\text{C}$ .

### SCHEME 1



The suspension containing 25 mmol or 1.85 g of  $\text{Ca}(\text{OH})_2$  (96 wt % pure with 3 wt % maximum  $\text{CaCO}_3$ , from Goodrich Chemical Enterprise) powder in 250 mL of DI water was heated to reaction temperatures (30, 45, 60, 80, and  $95^\circ\text{C}$ ) with stirring at 400 rpm for about 1 h. The temperature was maintained within  $\pm 2^\circ\text{C}$ . To this suspension, a solution containing 15 mmol or 1.73 g of 85 wt %  $\text{H}_3\text{PO}_4$  (total inorganic and organic impurities 0.035%, from Merck) in 250 mL of DI water was added at a rate of 3.5 mL/min using a peristaltic pump.

A Radiometer IONCheck65 four-point probe was used to measure the electrical conductivity of solutions and reaction mixtures during the acid addition. It was calibrated using NaCl standard solution ( $1000 \mu\text{S}/\text{cm}$  at  $25^\circ\text{C}$ ) provided by the manufacturer, and the temperature compensation was done automatically. The conductivity of the  $\text{H}_3\text{PO}_4$  solution was  $7.31 \text{ mS}/\text{cm}$  at room temperature ( $22^\circ\text{C}$ ). The pH of the reaction mixture was monitored using a Jenway 3510 pH meter. The acid addition was stopped once the end point was reached when the conductivity started to increase. The reaction mixture was then stirred for 2 h at reaction temperature followed by cooling to room temperature and overnight settling of precipitates. The precipitates were then filtered, washed with distilled water (3 times), and oven dried at  $60^\circ\text{C}$ . It has repeatedly been shown that the end product (dried powders) of the above reaction scheme (Scheme 1) under the same reaction conditions is mildly carbonated HA.<sup>11,12</sup> Therefore, the results pertaining to structural, compositional, and morphological characterizations are not shown here.

### Results

Figure 1 shows the variation of conductivity ( $\kappa$ ) of the reaction mixture with time at various temperatures, and in the  $\kappa$  vs  $t$  plots, three distinct regions can be identified at all temperatures based on the rate of decrease of conductivity,  $-\text{d}\kappa/\text{d}t$  as indicated in Figure 1. The magnitude of  $-\text{d}\kappa/\text{d}t$  is proportional to the rate of decrease of ionic concentration, which in turn is proportional to the rate of precipitation depending on the type

**TABLE 1: Rate of Decrease of Conductivity ( $\text{mS cm}^{-1} \text{ min}^{-1}$ ) and Approximate Duration of Reaction in Different Regions Indicated in Figure 1**

temp ( $^\circ\text{C}$ )	region I		region II	
	$-\text{d}\kappa/\text{d}t$	duration <sup>a</sup> (min)	$-\text{d}\kappa/\text{d}t$	duration (min)
30	0.01386	50	0.3087	21
45	0.01028	51	0.2833	21
60	0.00823	52	0.2562	20
80	0.00611	59	0.2268	19
95	0.00572	60	0.2059	18

<sup>a</sup> The duration of region I or time at which the magnitude of  $-\text{d}\kappa/\text{d}t$  changed is calculated from the linear regression equations in both regions at the given temperature, and they are truncated to the nearest whole number.

and number of ions (composition) in the precipitating compound. The magnitude of  $-\text{d}\kappa/\text{d}t$  may be the same for a compound with large numbers of ions and a low precipitation rate compared with a compound with fewer ions and a high precipitation rate. The variation in conductivity of the reaction mixture is also a function of the acid addition rate. However, this parameter is kept constant at all the reaction temperatures in the present study to eliminate its effect on the conductivity. The magnitudes of  $-\text{d}\kappa/\text{d}t$  in region I and region II are given in Table 1. As observed in Figure 1, the magnitude of  $-\text{d}\kappa/\text{d}t$  increased as the reaction progressed from region I to region II and the width of region I is greater than that of region II. In general, region I can be attributed to a nucleation dominant stage and region II to a growth dominant stage. In both regions, as the temperature is increased the magnitude of  $-\text{d}\kappa/\text{d}t$  decreases, indicating that the overall precipitation rate of HA decreases with increasing temperature. However, the gradual decrease in the magnitude of  $-\text{d}\kappa/\text{d}t$  in region I with increasing temperature suggests that the nucleated compound is, most likely, the same at all temperatures. The conductivity in region III is after the acid addition and there is no noticeable change in conductivity with time, indicating that the final product is reached just after the end point of the reaction.

The conductivity of the  $\text{Ca}(\text{OH})_2$  suspension before the acid addition decreases with increasing temperature. This is expected because of retrograde solubility of this sparingly soluble base in water.<sup>13</sup> It is reported that the solubility product,  $k_{\text{sp}}$ , of  $\text{Ca}(\text{OH})_2$  at  $25^\circ\text{C}$  is  $6.6 \times 10^{-6}$  and that the heat of dissociation,  $\Delta H_r$ , is  $-17.88 \text{ kJ}/\text{mol}$  for the reaction  $\text{Ca}(\text{OH})_2 \rightarrow \text{Ca}^{2+}(\text{aq}) + 2\text{OH}^{-}(\text{aq})$ .<sup>14</sup> Thus, using the vant Hoff equation, shown in eq 1

$$\ln \frac{k_{\text{sp}}}{k_{\text{sp}}^{25}} = \frac{\Delta H_r}{R} \left( \frac{1}{298} - \frac{1}{T} \right) \quad (1)$$

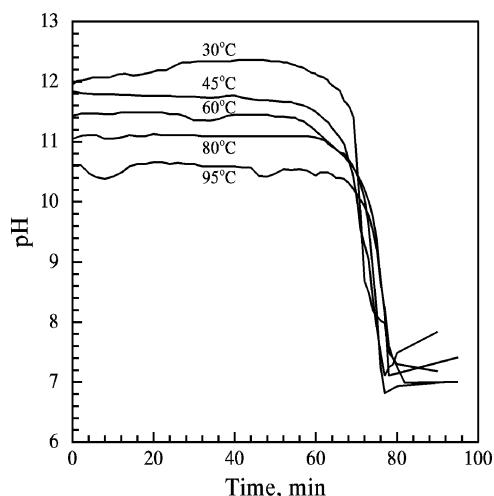
the  $k_{\text{sp}}$  of  $\text{Ca}(\text{OH})_2$  at other temperatures may be calculated using  $R$  as the universal gas constant ( $8.314 \text{ J}/(\text{mol K})$ ) and  $T$  as the absolute temperature. The calculated  $k_{\text{sp}}$ 's and other related measurements at different reaction temperatures are given in Table 2.

The poor solubility of  $\text{Ca}(\text{OH})_2$  in water leads to a large quantity of undissolved base before the acid addition, which dissolves as and when the  $\text{Ca}^{2+}$  ions and/or  $\text{OH}^{-}$  ions are consumed (due to phase nucleation and/or neutralization respectively by the acid). Therefore, the concentration of  $\text{Ca}^{2+}$  and  $\text{OH}^{-}$  ions in the solution is maintained until the reservoir of undissolved  $\text{Ca}(\text{OH})_2$  is completely consumed. This was experimentally verified by noting the variation in pH during the acid addition. The pH started to decrease abruptly only

**TABLE 2: Calculated and Measured Parameters of the Ca(OH)<sub>2</sub> Suspension at Different Temperatures**

temp (°C)	$k_{sp}, \times 10^{-6}$	Ca <sup>2+</sup> ions <sup>a</sup> (mmol)	$\kappa$ (mS/cm)	duration of constant pH (min)	
				obsd	est <sup>b</sup>
30	5.86	2.84	7.37	61	62
45	4.19	2.54	6.18	63	64
60	3.09	2.29	5.10	64	65
80	2.14	2.03	3.99	67	67
95	1.67	1.87	3.46	69	68

<sup>a</sup> Quantity of Ca<sup>2+</sup> ions in 250 mL of DI water. <sup>b</sup> Truncated to nearest whole number.

**Figure 2.** Variation of measured pH with time during the H<sub>3</sub>PO<sub>4</sub> addition to Ca(OH)<sub>2</sub> suspension.

toward the end of acid addition, and until then it remained more or less at the initial pH value followed by a slow decrease at the given temperature (Figure 2).

The dissolution of undissolved Ca(OH)<sub>2</sub> may take place due to (1) the increase in volume of water, (2) neutralization, and/or (3) phase nucleation as the acid addition is continued. The time taken for complete dissolution of Ca(OH)<sub>2</sub> can be estimated from eq 2 provided that only neutralization takes place. It was assumed that the dissolution rate of Ca(OH)<sub>2</sub> is not a rate-limiting step in deriving these equations, which is reasonable because the dissolution rate of Ca(OH)<sub>2</sub> is very high.<sup>13</sup> The first part of the right-hand side of eq 2 represents the dilution effect (adding acid) and the second part represents neutralization

$$[\text{Ca(OH)}_2] = \frac{\left(\frac{k_{sp}}{4}\right)^{1/3} (250 + f_r t)}{1000} + \frac{3}{2} C f_r t \quad (2)$$

where [Ca(OH)<sub>2</sub>] is the total dissolved quantity of Ca(OH)<sub>2</sub>,  $f_r$  is the acid addition rate (3.5 mL/min),  $t$  is time, and  $C$  is the concentration of H<sub>3</sub>PO<sub>4</sub> (0.06 mmol/mL). The factor of 3/2 is due to the stoichiometry of the reagents, 3 units of H<sup>+</sup> ions and 2 units of OH<sup>-</sup> ions. The computed times taken for complete dissolution, that is when [Ca(OH)<sub>2</sub>] is equal to 25 mmol, are given in Table 2.

## Discussion

The close agreement between the observed and estimated times at which the pH of the reaction mixture started to decrease

indicates that the dissolution of reservoir Ca(OH)<sub>2</sub> took place at a rate proportional to that of acid addition. This leads to an increase in the concentration of Ca<sup>2+</sup> ions as well as that of PO<sub>4</sub><sup>3-</sup> ions because of dissolution and acid addition, respectively. Therefore, if there is no phase nucleation, an increase in conductivity with time should be expected. However, we observed a decrease in conductivity with time (Figure 1). The decrease in conductivity could be due to (1) mixing of electrolytes and (2) dilution and phase formation.

It is reported that the conductivity of a solution containing both NaCl and KCl at a given total molar concentration (e.g., 0.01 M) is higher than that of the 0.01 M NaCl solution and lower than that of the 0.01 M KCl solution.<sup>15</sup> The conductivity of the NaCl solution is lower than that of the KCl solution. In our case, the conductivity of the Ca(OH)<sub>2</sub> suspension is higher than that of the H<sub>3</sub>PO<sub>4</sub> solution at 30 °C. Superficially, because of the difference in initial conductivity of the solutions, the theory of mixing of electrolytes may seem to explain the observed low magnitude of  $-d\kappa/dt$  in region I (Figure 1). However, a careful analysis, taking into consideration the types of electrolytes used, indicates that this may not be so just because H<sub>3</sub>PO<sub>4</sub> is a weak electrolyte. The pH of 0.06 M H<sub>3</sub>PO<sub>4</sub> solution (concentration used in this study) is about 1.85, which indicates only about 24% dissociation of H<sub>3</sub>PO<sub>4</sub> into H<sup>+</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup> ions, and the degree of dissociation is likely to increase in basic solutions. Therefore, it is considered to be a weak electrolyte, and its conductivity would be greater in fully dissociated conditions, which are likely in the present study, because of the high pH (pH > 10.5). Mason and Culvern reported a conductivity of 8.25 and 6.65 mS/cm at 25 °C for 0.07 and 0.05 M H<sub>3</sub>PO<sub>4</sub> solutions, respectively.<sup>16</sup> We measured a conductivity of 7.31 mS/cm for 0.06 M H<sub>3</sub>PO<sub>4</sub> solution, which is in good agreement with their results. They have also reported conductivities for 0.05152 and 0.06864 M NaH<sub>2</sub>PO<sub>4</sub> solutions to be 3.55 and 4.60 mS/cm, respectively, and our conductivity measurement for 0.06 M/0.01 M Na<sub>3</sub>PO<sub>4</sub>/NaOH solution is 11.73 mS/cm. This higher conductivity indicates a complete dissociation of Na<sub>3</sub>PO<sub>4</sub> into Na<sup>+</sup> and PO<sub>4</sub><sup>3-</sup> ions and that the PO<sub>4</sub><sup>3-</sup> ions were stable; that is, they did not form H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and/or HPO<sub>4</sub><sup>2-</sup> ions. It has been reported that the limiting molar conductivity (conductivity per unit mole of ions at extreme dilution where interactions between ions can be neglected) of H<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, OH<sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, HPO<sub>4</sub><sup>2-</sup>, and PO<sub>4</sub><sup>3-</sup> ions is 349.6, 50.1, 119.0, 199.1, 57.0, 66.0, and 207.0 S cm<sup>2</sup>/mol ((or mS/cm)/(mol/L)), respectively.<sup>17</sup> Therefore, the conductivity of H<sub>3</sub>PO<sub>4</sub> solution is expected to be much higher than 11.73 mS/cm if it completely dissociates. Also, as the addition of H<sub>3</sub>PO<sub>4</sub> to Ca(OH)<sub>2</sub> suspension continues, the concentration of OH<sup>-</sup> ions does not change (eq 2), but the concentration of Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> (rather than H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) ions increases with time. This can only lead to an increase in conductivity rather than to the observed decrease in conductivity with respect to time—at least in region I. Therefore, it leads us to consider another possibility for the decrease in conductivity with time, phase nucleation.

For the phase to nucleate, the degree of supersaturation,  $S_{CaP}$ , with respect to that phase should be higher and the phase should be thermodynamically as well as kinetically stable. Most often, the literature cites DCPD, OCP, and ACP as the precursor phases, depending on the pH and on the concentration of reagents, and HA is the most stable calcium phosphate compound at a pH greater than 8.<sup>5–10</sup> We excluded the formation of DCPD in the present study because the pH of the reaction mixture is greater than 10.5 and because DCPD forms only when

**TABLE 3: Calculated Solubility Product and Ionic Activity Product at the Beginning of Acid Addition and Dissociation Constant of Water at Different Temperatures<sup>b</sup>**

temp (°C)	$k_w, \times 10^{-13}$	IAP at $t = 1$ min			$k_{sp}$		
		OCP, $\times 10^{-33}$	ACP <sup>a</sup> , $\times 10^{-15}$	HA, $\times 10^{-26}$	OCP	ACP	HA
30	0.148	0.336	1.325	6.302	$1.20 \times 10^{-48}$	$1.60 \times 10^{-28}$	$2.84 \times 10^{-59}$
45	0.407	0.719	1.006	3.549	$1.64 \times 10^{-51}$	$4.12 \times 10^{-32}$	$1.82 \times 10^{-59}$
60	0.955	1.267	0.752	1.970	$4.07 \times 10^{-54}$	$2.24 \times 10^{-35}$	$8.93 \times 10^{-60}$
80	2.512	2.160	0.500	0.892	$3.02 \times 10^{-57}$	$2.68 \times 10^{-39}$	$2.43 \times 10^{-60}$
95	4.571	2.739	0.363	0.484	$2.27 \times 10^{-59}$	$5.84 \times 10^{-42}$	$7.27 \times 10^{-61}$

<sup>a</sup> We assumed that the phase is ACP though there is no experimental evidence for its existence in the present study. However, in the literature it has often been found and proven that one of the precursor phases is ACP. <sup>b</sup> URL <http://dbr.csoft.net/chem/bufhow.php>.

the pH is below 6.7.<sup>8</sup> The  $S_{CaP}$  can be calculated using eq 3

$$S_{CaP} = \frac{IAP - k_{sp}}{k_{sp}} \quad (3)$$

where IAP is the ionic activity product which is given below for different phases

$$IAP_{HA} = a_1^5 a_2^3 a_3 \quad (4)$$

$$IAP_{OCP} = a_1^4 a_2^3 k_w / a_3 \quad (5)$$

$$IAP_{ACP} = a_1^3 a_2^2 \quad (6)$$

where 1, 2, and 3 represent  $Ca^{2+}$ ,  $PO_4^{3-}$ , and  $OH^-$  ions, respectively,  $k_w$  is the dissociation constant of water,  $a_i (=c_i\gamma_i)$  is the activity,  $c_i$  is the concentration, and  $\gamma_i$  is the activity coefficient of the ion  $i$ .

The activity coefficient of ions is calculated using the Debye–Huckel equation (eq 7)

$$\log \gamma_i = -\frac{Az_i^2\sqrt{\mu}}{1 + B\alpha_i\sqrt{\mu}} + C\mu \quad (7)$$

$$A = \frac{1}{4\pi \ln 10} \left( \frac{e^2}{\epsilon k_B T} \right)^{3/2} \left( \frac{\rho N_A}{2} \right)^{1/2} \quad (7a)$$

$$B = 2 \left( \frac{e^2}{\epsilon k_B T} \right)^{1/2} \left( \frac{\rho N_A}{2} \right)^{1/2} \quad (7b)$$

$$\mu = \frac{1}{2} \sum_i c_i z_i^2 \quad (7c)$$

$$\epsilon_r = 78.54 - 0.361(T - 298) + 6.91 \times 10^{-4}(T - 298)^2 \quad (7d)$$

where  $A$  and  $B$  are temperature dependent theoretical constants,  $\alpha$  is the hydrated ionic radius (6.0, 3.5, and 4.0 Å for  $Ca^{2+}$ ,  $OH^-$ , and  $PO_4^{3-}$  ions, respectively<sup>18</sup>),  $C$  is the ionic system specific constant (0.055 in this case), and  $\mu$  is the ionic strength. The other terms are given as follows:  $e$  is the electronic charge,  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature,  $\rho$  is the density of water,  $N_A$  is the Avogadro number,  $\epsilon (= \epsilon_0 \epsilon_r)$  is the dielectric constant,  $c$  is the concentration, and  $z$  is the charge of the ions. The relationship between temperature and  $\epsilon_r$  for water is given in eq 7d.<sup>19</sup>

The temperature dependence of the solubility product of the calcium phosphates in the temperature range used in this study is not available. However, it is available in the temperature range 5–37 °C (eq 8) for HA,<sup>20</sup> and it is assumed that eq 8 is applicable to higher temperatures

$$\log k_{sp} = -8219.41T^{-1} - 1.6657 - 0.09821T \quad (8)$$

For OCP, the  $k_{sp}$  at 25 °C is  $1.25 \times 10^{-47}$  and at 37 °C it is  $5.1 \times 10^{-50}$ ,<sup>21,22</sup> and for ACP it is  $3.02 \times 10^{-27}$  at 25 °C and  $6.31 \times 10^{-26}$  at 20 °C.<sup>23,24</sup> With the use of these data and eq 1,  $k_{sp}$  values at the temperatures used in this study for OCP and ACP were calculated. They are listed in Table 3 along with the  $k_{sp}$  of HA calculated using eq 8. The  $k_{sp}$  values of HA at different temperatures calculated using eq 1 ( $6.3 \times 10^{-59}$  at 25 °C and  $2.35 \times 10^{-59}$  at 37 °C<sup>21,22</sup>) differed only in factor but not by the order of magnitude from those calculated using eq 8. Therefore, the  $k_{sp}$  values calculated for OCP and ACP can be considered valid, considering the lack of availability of data and the very high  $S_{CaP}$  of the reaction mixtures. Because of the retrograde solubility of calcium phosphates, the  $S_{CaP}$  increases with increasing temperature and it is highest for HA.

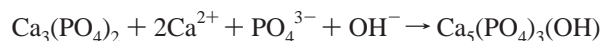
Equations similar to eq 2 can be derived to estimate the dissolved quantity of  $Ca(OH)_2$  if phase formation takes place from the beginning of acid addition. Thus, if ACP is the first compound formed from the beginning of acid addition, it can be calculated from eq 9

$$[Ca(OH)_2] = \frac{\left(\frac{k_{sp}}{4}\right)^{1/3} (250 + f_r t)}{1000} + \frac{3}{2} C f_r t \quad (9)$$

Note that eqs 2 and 9 are exactly the same and that this is because the factor 3/2 in eq 9 now represents the stoichiometry of ACP (3 units of  $Ca^{2+}$  and 2 units of  $PO_4^{3-}$  ions). Obviously, eq 9 predicts the same pH values and time taken for the complete dissolution of  $Ca(OH)_2$  as eq 2 does. In addition, it predicts that the concentration of  $Ca^{2+}$  ion does not change with time, as the quantity of  $H^+$  and  $PO_4^{3-}$  ions added would exactly balance the dissolved quantity of  $Ca(OH)_2$  by neutralization and by forming ACP. As the addition of acid progresses, the concentration of ACP, and hence its interaction with ions, would increase which in turn would decrease the conductivity at a rate proportional to its formation. Thus, the formation ACP in region I explains the observed results in Figures 1 and 2.

In region II, when ACP (5 ions per molecule) transforms into crystalline HA (9 ions per molecule), it uptakes 4 units of ions for every unit of ACP. The ACP → HA transformation reaction in the ionic medium may be given by Scheme 2 as the acid is continuously added to the reaction mixture until the end of region II.

## SCHEME 2



Because of the consumption of large quantities of ions, it is reasonable that the magnitude of  $-dk/dt$  was higher in region



II than that in region I and it is more likely that the transformation will occur via reaction Scheme 2 in the present study.

If HA is the first compound formed, then the dissolved quantity of  $\text{Ca}(\text{OH})_2$  can be estimated from the relationship shown in eq 10

$$[\text{Ca}(\text{OH})_2] = \frac{\left(\frac{k_{\text{sp}}}{4}\right)^{1/3} (250 + f_r t)}{1000} + \frac{5}{3} C f_r t \quad (10)$$

$$[\text{OH}^-] = 2[\text{Ca}(\text{OH})_2] - \frac{1}{3} C f_r t \quad (10a)$$

Equation 10 predicts a higher dissolution rate compared to that of eq 2; for example, the time taken for complete dissolution calculated using eq 10 is 57 and 61 min at 30 and 95 °C, respectively, and therefore, it predicts the pH of the solution would start to decrease earlier than that predicted by eq 2 or the experimentally observed time. However, the pH would still be maintained at the initial value in the reaction mixture until complete dissolution of  $\text{Ca}(\text{OH})_2$  or up to the computed time (eq 10a). Again, the factor 1/3 in eq 10a is due to the stoichiometry of HA (3 units of  $\text{PO}_4^{3-}$  ions and 1 unit of  $\text{OH}^-$  ion). It should be noted that according to eq 2 the dissolution of  $\text{Ca}(\text{OH})_2$  is governed by the concentration of  $\text{OH}^-$  ions and that according to eq 10 the dissolution is governed by the concentration of  $\text{Ca}^{2+}$  ions. This situation may lead to confusion if equations to compute the concentration of  $\text{OH}^-$  ions are derived from eq 2 and eq 10, as both give essentially the same expressions for the concentration of  $\text{OH}^-$  ions. Since the dissolution of the reservoir of  $\text{Ca}(\text{OH})_2$  acts as a source for both  $\text{Ca}^{2+}$  and  $\text{OH}^-$  ions, the focus should be on eqs 2, 9, 10, and similar ones, rather than on the expressions for concentrations of individual ions. As mentioned earlier, according to eq 10 the time at which  $\text{Ca}(\text{OH})_2$  completely dissolves is a few minutes earlier than that observed or calculated using eq 2. Moreover, if HA were nucleated from the beginning of acid addition, the IAP with respect to HA would be maintained at a constant value until the complete dissolution of  $\text{Ca}(\text{OH})_2$  at the given temperature and would only decrease after that. Therefore, a lower magnitude of  $-\text{d}\kappa/\text{d}t$  should be expected in region II than in region I. However, this is not observed, as is evident from Figure 1 and Table 1. Therefore, it is unlikely that the nucleation of HA took place from the beginning of acid addition.

Similarly, for OCP formation, the dissolved quantity of  $\text{Ca}(\text{OH})_2$  may be expressed as shown in eq 11

$$[\text{Ca}(\text{OH})_2] = \frac{\left(\frac{k_{\text{sp}}}{4}\right)^{1/3} (250 + f_r t)}{1000} + \frac{4}{3} C f_r t \quad (11)$$

$$[\text{OH}^-] = 2[\text{Ca}(\text{OH})_2] + \frac{8}{3} C f_r t \quad (11a)$$

Clearly, eq 11 predicts a slower dissolution rate compared to eq 2 and an increase in pH or an increase in  $\text{OH}^-$  ion concentration with time (eq 11a), suggesting an increase in conductivity with time. Again, from Figures 1 and 2, it can be said that OCP was not the phase that nucleated at the beginning of acid addition. Moreover, this phase is not stable in basic media, as reported by several others.<sup>5–10</sup>

It therefore follows, from the conductivity data and the pH values, that the precursor compound should be free of both  $\text{H}^+$  and  $\text{OH}^-$  ions. In other words, a low magnitude of  $-\text{d}\kappa/\text{d}t$  in region I may be explained if the  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ion associate

and form a precursor phase such as ACP for HA. A number of studies have shown that the formation of HA proceeds via ACP in fairly high supersaturated solutions.<sup>5–10</sup>

In previous experiments carried out to study the kinetics of  $\text{ACP} \rightarrow \text{HA}$  transformation, ACP was synthesized and then the synthesized ACP was allowed to transform into HA under various solution conditions. It was shown that the transformation proceeds with the initial induction period (over 100 min at a pH of 12)<sup>7</sup> where the extent of transformation is minimal or not significant, followed by a rapid transformation (proliferation period) and a gradual “tapering off” period, resulting in sigmoid kinetic curves. The induction period is a result of the requirement in solution to reach a critical degree of supersaturation for the HA to nucleate.<sup>5</sup> This type of kinetic curve is typical of solid-state reactions involving nucleation and autocatalytic transformation. The induction period was shown to increase and the rate of transformation was shown to decrease with increasing pH in the range 7–10,<sup>5,7</sup> with decreasing pH at values greater than 10.25,<sup>7,9</sup> with decreasing initial  $\text{Ca}^{2+}$  ion concentration in solution,<sup>9</sup> and with decreasing temperature.<sup>10</sup> The induction period also increases with increasing ACP slurry concentration; however, the rate of  $\text{ACP} \rightarrow \text{HA}$  transformation is not affected.<sup>5</sup> A longer induction period indicates a higher stability of ACP, and factors that increase these periods would also increase the stability of ACP. The present work differs from the above transformation kinetic studies in that the critical degree of supersaturation is achieved here from external sources (by addition of acid and dissolution of the  $\text{Ca}(\text{OH})_2$  reservoir) rather than from the dissolution of precipitated ACP itself. Therefore, strictly speaking, in the present work there is no induction period and region I represents the ACP formation step, whose rate of formation is affected by its stability.

Because of the nature of the reagents used, we encountered parameters which acted together to retard the rate of ACP formation. As the temperature is increased, the concentration of  $\text{Ca}^{2+}$  ion and the pH in the reaction mixture decrease. Whereas the former decreases the stability of ACP, the latter increases it. Therefore, it can be said that the overall effect of temperature led to a decreased rate of formation of ACP, and consequently, we observed the magnitude of  $-\text{d}\kappa/\text{d}t$  to decrease with an increase in temperature (Table 1). The same argument is applicable for the variation of  $-\text{d}\kappa/\text{d}t$  in region II with temperature as a factor increasing the stability, decreasing the rate of transformation. We also observed that the duration of region I increases with increasing temperature (Table 1). The duration of region I is shorter (about an average of 10 min) than the time in which the pH started to decrease (Tables 1 and 2), indicating the  $\text{ACP} \rightarrow \text{HA}$  transformation starts before the complete dissolution of  $\text{Ca}(\text{OH})_2$ . Also, a sharp change in slope from region I to region II and no further change in slope in region II indicates that no intermediate compound is formed during the  $\text{ACP} \rightarrow \text{HA}$  transformation. In other words, the transformation reaction started and continued for a considerable amount of time, with the acid addition, at the respective initial pH and  $\text{Ca}^{2+}$  ion concentration in the reaction mixture at the given temperature. Also, because of the autocatalytic nature of this transformation, the formation of ACP in region II is highly unlikely. Therefore, the factor that determines the time at which the  $\text{ACP} \rightarrow \text{HA}$  transformation step starts is the amount of ACP formed. According to the criteria proposed for the onset of the proliferation period,<sup>5</sup> combinations of ACP slurry and initial  $\text{Ca}^{2+}$  ion concentrations may be found to increase the induction time with increasing temperature. It seems that such combinations in the present study have led to the increased duration of

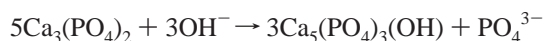
region I with temperature changes. For example, after 50 min of acid addition, the ACP concentration in the reaction mixture will be about 12.35 mmol/L. At 30 °C, this ACP concentration was sufficient for the ACP → HA transformation to start. However, at 95 °C, it was not sufficient because of low pH and Ca<sup>2+</sup> ion concentration in solution, and therefore, ACP continued to form until after about 60 min of acid addition (Table 1).

As mentioned earlier, in region II, the ACP → HA transformation reaction occurs via reaction Scheme 2. If this is the case, the maximum quantity of ACP should not exceed 5 mmol, as estimated from the initial quantity of reagents and the stoichiometric ratio of Scheme 2. The total quantity of ACP present at the onset of region II is calculated, from the total quantity of PO<sub>4</sub><sup>3-</sup> ion added to the reaction mixture until the end of region I (Table 1), to be 5.250, 5.355, 5.460, 6.195, and 6.300 mmol at 30, 45, 60, 80, and 95 °C, respectively. Clearly, the calculated total ACP formed until the end of region I is slightly higher than 5 mmol and increases with increasing temperature. This increase cannot be ignored as a simple artifact or experimental error as the concentration of H<sub>3</sub>PO<sub>4</sub> used for all the reaction is from the same stock solution of 0.06 M and the acid addition rate is also the same. Therefore, it is likely that the ACP → HA transformation took place via more than one route (Schemes 3 and 4) and that Scheme 2 is the most predominant as explained above

### SCHEME 3



### SCHEME 4



It is worth noting that the duration of region II decreases very little, about 3 min when the temperature is raised from 30 to 95 °C, while the total duration of the reaction until the end point, that is, the sum of regions I and II (Table 1) and the time taken for complete dissolution (average of observed and calculated in Table 2), increases about 7 min with the same increase in temperature. The shorter duration of region II may be due to the fact that the transformation reaction continued in rapidly decreasing pH, after the complete dissolution of Ca(OH)<sub>2</sub> in the reaction mixture. Also, with the higher quantity of ACP and rapidly decreasing pH at higher temperatures, the proportion of transformation routes other than Scheme 2 might have increased and hence led to a higher conductivity at the end point of the reaction. This could also be a reason for the decreased transformation rate. It is also worth mentioning that the values of  $-dk/dt$  in region II represents the transformation rate only up to the end point of the reaction. The remaining ACP and its transformation (if any) beyond the end point are not included. Nevertheless, any phase transformation beyond the end point will not substantially affect the overall transformation kinetics, and only the proliferation period or region II can be subjected to kinetic analysis.<sup>5</sup> Thus, our results show that the ACP →

HA transformation rate decreased with increasing temperature in the reaction system considered here.

### Conclusions

Hydroxyapatite was precipitated using sparingly soluble reagents, namely, calcium hydroxide and orthophosphoric acid, at different temperatures up to 95 °C. The precipitation process was monitored by measuring the solution conductivity and pH. The HA precipitation proceeded through a precursor phase with a molecular formula of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. Both the rate of formation of this precursor phase and its transformation rate to crystalline HA decreased with increasing temperature. The sparingly soluble nature of calcium hydroxide, its retrograde solubility with temperature, and the continual addition of orthophosphoric acid at a fixed rate seemed to affect the overall reaction kinetics. The very nature of the experimental procedure made this system of reaction unique and different from traditionally adopted experimental procedures as far as the phase transformation and kinetics are concerned.

**Supporting Information Available:** X-ray diffractograms, FTIR spectra, and FESEM micrographs. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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