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A Novel Method for the Simultaneous, Titrant-Free Control of pH and Calcium Phosphate Mass Yield

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ABSTRACT

Calcium phosphate (CaP) bioceramics have long been of interest for the unique properties that they exhibit as bone substitute materials. By harnessing the unique bone-bonding capacity of CaP's, biomaterials scientists have made great strides over the past 2 decades to produce novel materials to assist in the treatments of defects caused by trauma, disease, or both. In recent years, however, it has become apparent that the traditional set of techniques used to produce calcium phosphates does not satisfy all of the requirements necessary to meet the challenges of emerging applications. In particular, recent interest in (i) the synthesis of coprecipitated CaP/bioorganic composites and (ii) the investigation of the mechanisms of biomineralization has highlighted the need for new methods to control pH and CaP mass yield.

Introduction

Calcium phosphates (CaP's) have long been the focus of extensive research for their potential use in biological systems.[1–3] The range of products in which they can be found includes fertilizers, adsorbents for chromatography, and drug delivery systems. But by far the most widely recognized application of calcium phosphates is their use as bone substitutes, which over the past 3 decades has yielded a lucrative commercial market currently estimated in excess of $400 million (U.S.) per year worldwide.

The clinical success of calcium phosphates as orthopedic biomaterials is due in no small part to the unique capacity that they impart to implants: the ability to interact with the surrounding bone. The first widespread application of calcium phosphates (plasma-sprayed CaP coatings for metallic prostheses[4–6]) provides a classic example of this capacity, as the advent of these coatings imparted to metallic surfaces previously inert to the human body the ability to bond directly to bone. The past 15 years have witnessed the emergence of a vast number of other CaP-based products, including dense bone fillers,[7–9] porous implants,[10,11] advanced composites,[12,13] and bone cements,[14,15] all of which aim to exploit this same unique property.

Table 1. Ca/P Ratios and Chemical Formulas for Various Calcium Phosphate Bioceramics

Ca/P ratio	compound	formula
0.50	monocalcium phosphate monohydrate (MCMP)	$Ca(H_2PO_4)_2{\cdot}H_2O$
0.50	monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$
1.00	brushite (DCPD)	$CaHPO_4{\cdot}2H_2O$
1.00	monetite (DCPA)	$CaHPO_4$
1.33	octacalcium phosphate (OCP)	$Ca_8(H_2PO_4)_2(PO_4)_4{\cdot}5H_2O$
1.50	amorphous calcium phosphate (ACP)	$Ca_3(PO_4)_2{\cdot}xH_2O$
1.50	α-tricalcium phosphate (α-TCP)	$\alpha\text{-}Ca_3(PO_4)_2$
1.50	β-tricalcium phosphate (β-TCP)	$\beta\text{-}Ca_3(PO_4)_2$
1.67	hydroxyapatite (HAp)	$Ca_{10}(PO_4)_6(OH)_2$
2.00	tetracalcium phosphate (TTCP)	$Ca_4P_2O_9$

While the precise mechanisms underlying the bone-bonding capacity of calcium phosphates are still not fully understood, it is largely recognized that by controlling both (i) the precise chemical composition of the CaP species present and (ii) the mass fraction of each of these species, one can tailor the manner in which the resultant biomaterial interacts with its environment. Efforts aimed at controlling chemical composition have centered on development of production methods for pure forms of various calcium phosphates (Table 1), and recent initiatives have aimed to produce variants of some of these CaP's through the carefully controlled substitution of secondary ions.[16,17] Control of mass fraction, on the other hand, has traditionally been achieved by mechanical mixing of the selected phases. Although this approach has proven highly effective for traditional systems in which pre-fabricated CaP particles can be dry mixed (either with synthetic polymers or with other calcium phosphates), it has proven inadequate to meet the needs of some of the latest applications.

A major area of recent progress has been the production of CaP/bioorganic composites.[18–20] Interest in these materials stems from their compositional similarity to calcified tissue,[21] a biomimetic character of particular significance to the field of tissue engineering.[22,23] Recent developments have produced complexes with a high degree of chemical and nanostructural similarity to bone.[20] However, a significant deficiency of current synthesis

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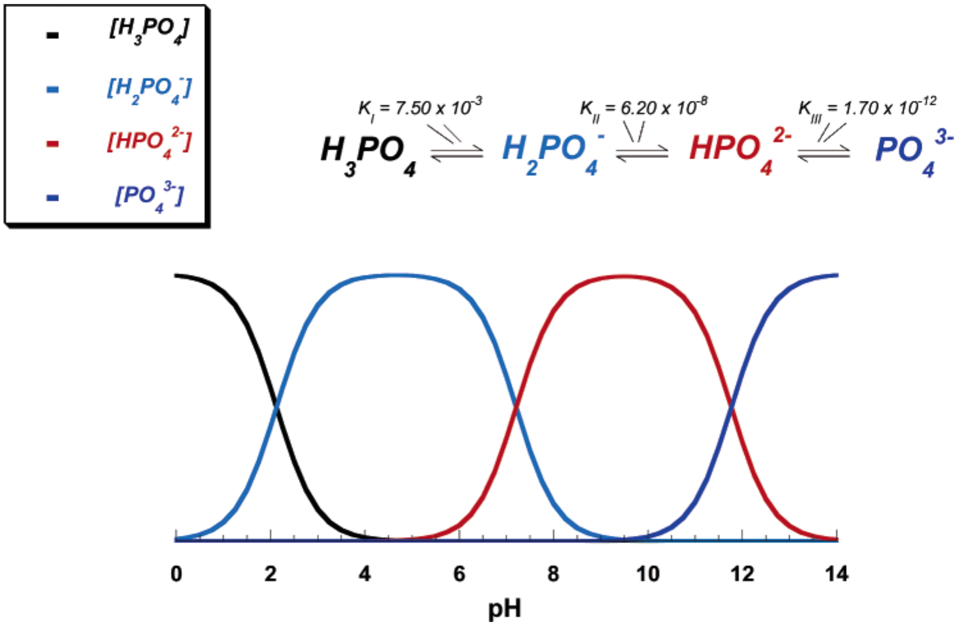

FIGURE 1. pH variation of ionic concentrations in tripotic equilibrium for phosphoric acid solutions.

methods is the lack of systematic means through which the CaP content, and hence the CaP/bioorganic ratio, can be controlled.

Our group is currently engaged in research pertaining to all aspects of calcium phosphate biomaterials, from synthesis to commercialization to clinical application. Using this broad background as a base, we have developed a platform technique the applicability of which ranges from the investigation of biomineralization mechanisms to the development of novel CaP/bioorganic composites. Developed for the room-temperature synthesis of calcium phosphates, this simple technique enables the simultaneous control of pH and CaP mass yield without the addition of titrants. In this Account, we discuss the development of the technique in the context of the acidic CaP brushite. Application to the somewhat more complex system of hydroxyapatite and to specific examples of the production of CaP/bioorganic composites is left for future reports.

Synthesis of Novel CaP Biomaterials: Changing Requirements

The Importance of pH. The effect of pH on the synthesis of calcium phosphates is integrally linked to the properties of phosphate-containing solutions. Due to the tripotic equilibrium that exists within these systems, variations in pH alter the relative concentrations of the four polymorphs of phosphoric acid (Figure 1) and thus both the chemical composition and the amount of the CaP that forms by direct precipitation.[24] This complex equilibrium makes the control and prediction of CaP precipitation infinitely more difficult than, say, that of simple table salt from a NaOH/HCl solution, because the latter involves strong monoprotic acids and bases the ionic concentrations of which are vastly easier to predict.

During the formation of CaP/bioorganic complexes, pH plays a role in determining the properties of both the inorganic and organic phases. While its influence on the inorganic phase is largely compositional (chemical composition and mass fraction), altered pH induces changes in the organic phase that are generally structural with variations in water uptake,[25,26] alterations in helical structure,[27] and disruption of long-range order[28,29] all resulting from fluctuations in synthesis-time pH. Furthermore, pH can affect the solubility of bioorganic species during synthesis, a property particularly important for distinguishing between systems in which calcium phosphates and bioorganics are coprecipitated[20,24] and those in which the CaP species is merely precipitated onto an insoluble bioorganic.[30,31]

Mass Yield. As mentioned previously, traditional methods for producing CaP biomaterials have involved, almost exclusively, the prefabrication of monolithic calcium phosphates of the desired chemical composition, followed by mixing of the desired amounts of each. It is thus, perhaps, not surprising that considerations of mass yield have generally been paid little heed, because these methods permit excess CaP to be merely discarded.

For the production of CaP/bioorganic composites, however, these considerations take on a new significance. The CaP and organic components of these composites are generally formed simultaneously via a technique known as coprecipitation. This approach is often responsible for some of the more distinctive biomimetic features of the resulting biomaterials, including the nanometer size of their CaP crystallites and orientational relationships between their organic and inorganic constituents.[20,32] However, it also dictates that the CaP content of the precipitate is determined at the time of synthesis with no opportunity to discard unwanted quantities or add additional ones without significantly altering the properties of the material.

Contaminants. Similar to that of mass yield control, the issue of contaminant phases containing residual quantities of titrant species has traditionally not been a major focus of concern. Methods involving prefabrication of calcium phosphates all employ filtration and rinsing of precipitates before inclusion in the final product, thereby allowing titrant species to be eliminated while still in their aqueous state.

Efforts to produce porous forms of CaP/bioorganic composites have resulted in the emergence of freeze-drying as a preferred technique for producing scaffolds for tissue engineering.[33] Freeze-drying involves the controlled production of a frozen intermediate comprised of water (in the form of ice), the chosen bioorganic, calcium phosphate, and all residual reactants, solvents, and titrants present in the reaction mixture. Subsequent sublimation/evaporation steps remove most or all of the pure species (water, residual acids, and aqueous titrants), leaving a porous body containing only the CaP/bioorganic composite.

Since all titrants are incorporated within the frozen intermediate, however, titrants can sometimes react with each other or with reactant ions as freezing progresses, forming solid contaminant phases that persist in the final product even after the sublimation/evaporation steps. These phases can drastically alter the mechanical properties and solubility of the composite and can also reduce its ability to interact with the surrounding bone.[34]

The requirements highlighted above demonstrate that a technique simultaneously enabling (i) control of mass yield, (ii) regulation of pH, and (iii) minimization of titrant additions would be a welcome addition to the literature. While a number of techniques, including application of pH stat techniques via an automatic titrator, could be used to address the first two requirements, the third remains particularly difficult to satisfy. This situation is further exacerbated by the fact that risk of sodium contamination makes the use of NaOH as a titrant undesirable, leaving the volatile (and generally unpleasant) compound ammonia as the most common means for pH control. The simple solution to this problem lies not in the identification of novel titrating agents but rather in a reevaluation of the reactants themselves.

Simplest Is Best

Typically, methods to produce calcium phosphates employ a single calcium source, most often either calcium hydroxide[35] or calcium nitrate.[36] The dissociation products of these two compounds are drastically different in terms of their acidity or alkalinity. Calcium nitrate dissociates in water to a slightly acidic solution with a pH of approximately 5, whereas calcium hydroxide forms an equilibrium with deionized water around pH 12.

By using *both* of these compounds in varying proportions, solutions of controlled acidity/alkalinity can be produced, increased $Ca(OH)_2$ content leading to higher pH values and increased $Ca(NO_3)_2{\cdot}4H_2O$ content resulting in lower pH. This simple modification allows the pH of any calcium-containing solution to be controlled by effectively using the reactants themselves as titrants. pH can be varied both by changing the overall ion concentrations and by changing the nitrate-to-hydroxide ratio. Residual secondary ions are thus limited to the nitrate anion, which, in the absence of suitable cations, does not form any solid contaminant phases. This approach thus limits contaminant phases to species that can be removed by, for instance, the sublimation step of a freeze-drying process.

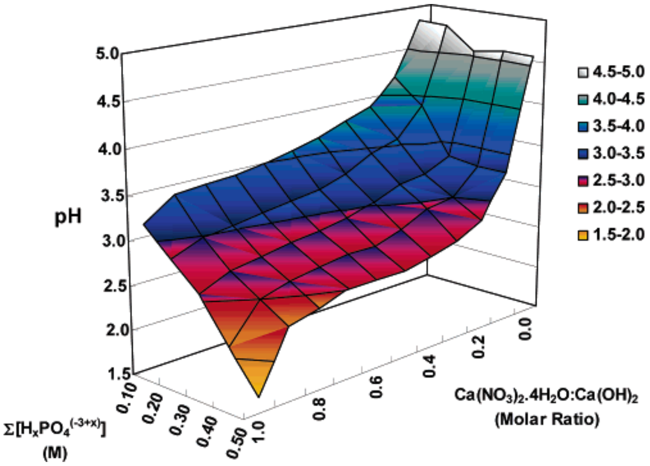

FIGURE 2. Map of pH variation for brushite synthesis (Ca/P = 1.00).

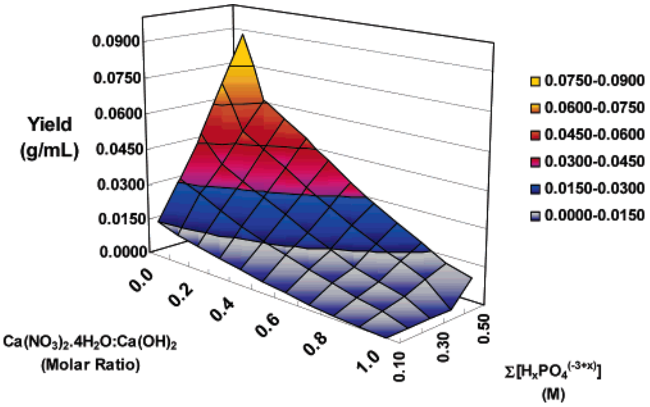

FIGURE 3. Map of variation in mass yield for brushite synthesis (Ca/P = 1.00).

By repeating the synthesis of a given CaP (brushite in the present case) at various combinations of reactant concentrations and nitrate/hydroxide ratios, one can produce a comprehensive map of pH variation at any given Ca/P ratio (Figure 2). Similarly, a map of mass yield (per milliliter of reactant solution) can be produced by weighing the filtered and dried products of synthesis at a given set of reactant conditions (Figure 3).

A Complex Balance

In searching for a means through which the observed variations in pH and mass yield can be predicted, controlled, or both, it is instructive to examine the variations in theoretical supersaturation at each combination of ion concentration and nitrate/hydroxide ratio observed.

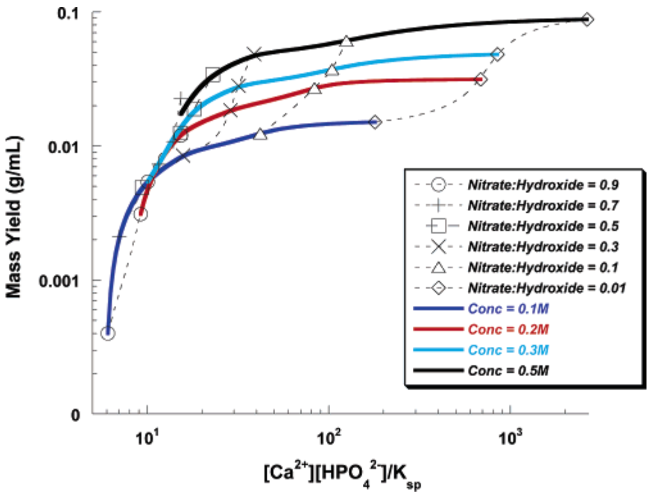

FIGURE 4. Variation of mass yield with supersaturation for various combinations of concentration and $Ca(NO_3)_2 \cdot 4H_2O/Ca(OH)_2$ ratio.

In the idealized case of a solution of which the ionic species are limited to the four polymorphs of phosphoric acid plus calcium (Ca^{2+}), it would be expected that the amount of brushite precipitated (i.e., mass yield) would be governed by the supersaturation coefficient. As can be seen from Figure 4, however, altering the nitrate/hydroxide ratio leads to behavior that decidedly does not conform to this ideal case.

The complex systematic variations in the relationship between mass yield and supersaturation shown in Figure 4 are most likely due to the effects of nitrate ions (NO_3^-) on the activities of the other ionic species present. While further investigation of this effect coupled with mathematical modeling techniques may provide a means through which the variations shown in Figures 2 and 3 can be quantified, use of the figures themselves allows us to forego such complex approaches to produce a powerful method for the control of both mass yield and pH.

Application: Titrant-Free Control of Synthesis Parameters

While the pH and mass yield maps shown in Figures 2 and 3 constitute useful tools in their own right, it is from their combined use that a technique to provide simultaneous control of pH and yield without the need for titrants emerges.

Control of Mass Yield at Constant pH. By identifying contours of constant pH on the map in Figure 2, one can identify the set of concentration–nitrate/hydroxide ratio combinations for which pH remains at a given level (Figure 5a). Because the axes for the mass yield map (Figure 3) are identical to those on the pH map, the trace of this contour can be transposed directly from one to the other (Figure 5b).

Selecting points of intersection between the transposed trace and the constant-mass contours subsequently allows the identification of constitutive relationships for both ion concentration and nitrate/hydroxide ratio (Figure 5b); use of these relationships enables the control of mass yield in a systematic manner, while maintaining pH at a constant level.

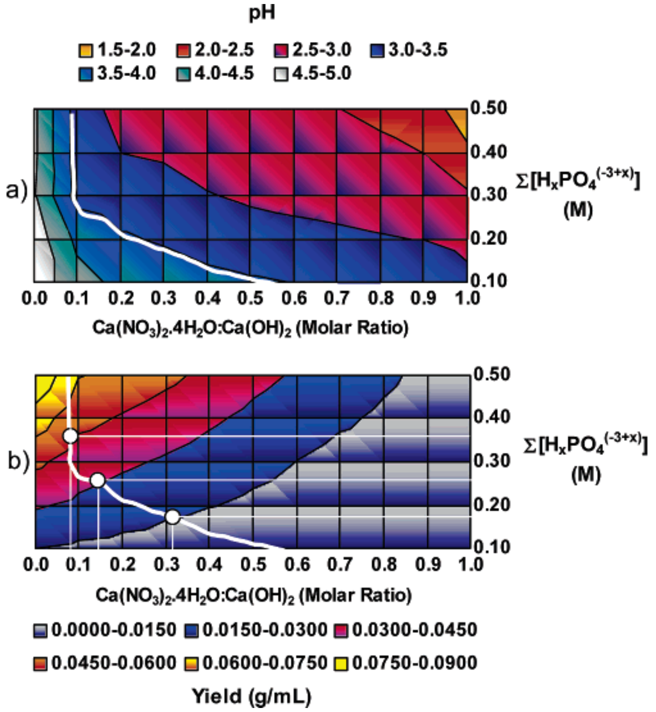

FIGURE 5. Application of pH and mass yield maps for titrant-free control of mass yield at constant pH.

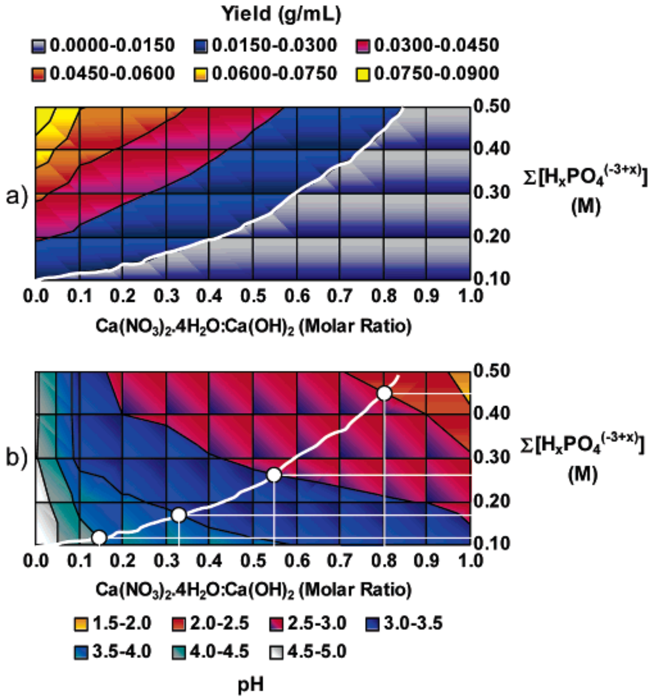

FIGURE 6. Application of pH and mass yield maps for titrant-free control of pH at constant mass yield.

Control of pH at Constant Mass Yield. Applying the same approach in reverse order (Figure 6) allows the transposition of constant-mass contours on the yield map onto the pH map, thereby permitting the control of pH at constant mass yield.

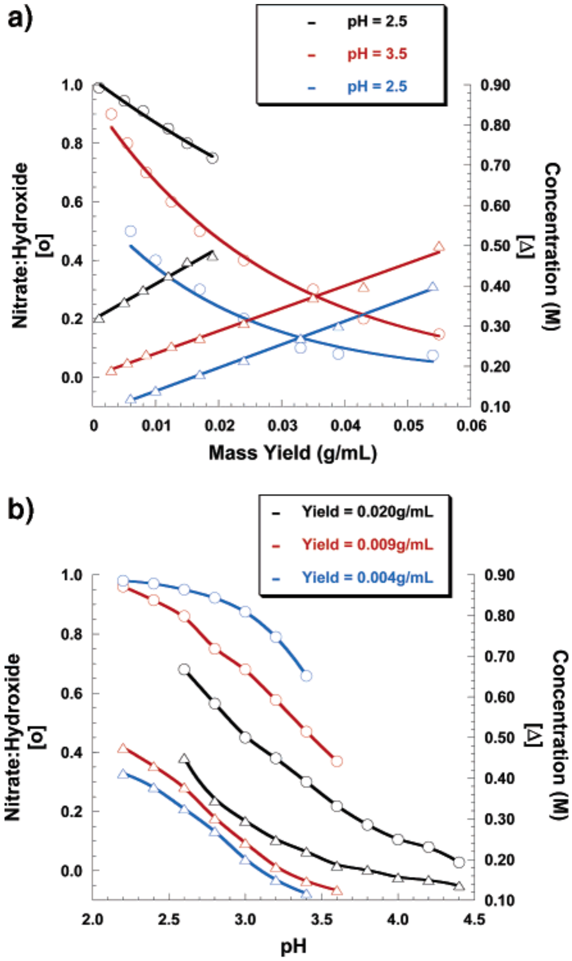

FIGURE 7. Selected concentration and nitrate/hydroxide ratio curves for (a) control of mass yield at constant pH and (b) control of pH at constant mass yield.

Further Implications. The constitutive relationships produced using these two techniques (Figure 7) are simple and can be executed with laboratory compounds that are both inexpensive (bearing in mind the minimum \$5000 (U.S.) price tag for a pH-stat autotitrator) and readily available. Furthermore, by varying the solution ratio of calcium to phosphorus and the temperature at which synthesis is carried out, one can achieve additional degrees of control. Higher calcium to phosphorus ratios, for instance, enable the extension of this technique (with some modification) to the synthesis of apatite, while elevated temperature allows the precipitation of monetite to be addressed. Efforts currently underway are focusing on the synthesis of amorphous calcium phosphate, a species for which reproducible synthesis methods are elusive at best.

Perhaps the most powerful potential applications of the techniques described here involve the precipitation of calcium phosphates onto bioorganics. Conventional means of controlling pH, using buffers or titrants, introduce additional contaminant ions into the reactant system that may alter both CaP precipitation behavior and the conformational structure of the bioorganic. In some cases, the presence of titrants can result in the precipitation of phases containing contaminant ions, and in extreme cases, such as when freeze-drying is involved, these contaminant phases can even prevent formation of the desired CaP species (Figure 8). As mentioned previously, the issue of controlling the CaP/bioorganic ratio has been largely ignored to date, most likely due to the lack of a viable means through which it can be addressed. Simple application of the platform technique described here has facilitated the development of a method for producing composition-controlled CaP/bioorgnanic composites the inorganic-to-organic ratio of which can be altered with degrees of accuracy foreign to techniques reported to date. Details of this and other studies will be reported in our future publications.

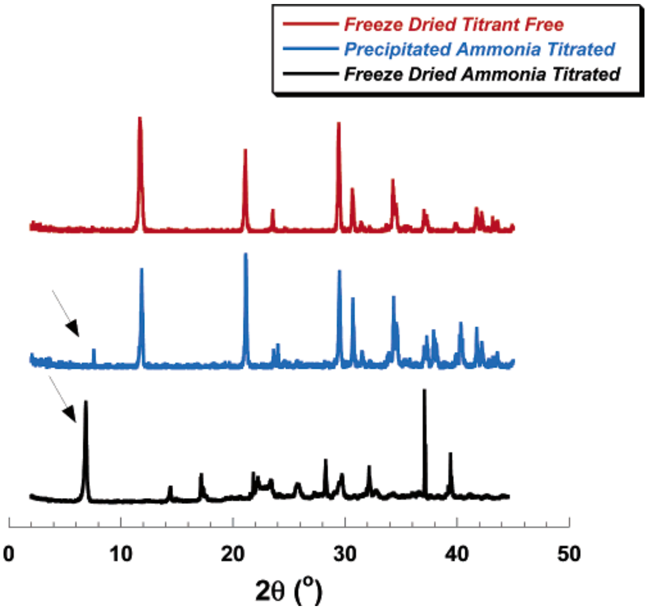

FIGURE 8. X-ray diffraction patterns for brushite synthesised at pH 3.2 using different synthesis techniques. Arrows indicate principle peak for the contaminant phase ammonium nitrate.

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