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ScienceDirect

Ceramics International 40 (2014) 2751–2761

CERAMICS INTERNATIONAL

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The effect of excess phosphate on the solubility of hydroxyapatite

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Received 2 October 2013; accepted 10 October 2013
Available online 18 October 2013

Abstract

The solubility of hydroxyapatite (HA) is critical in fields such as medicine, dentistry and geochemistry. Previously, it had been found that it was apparently slightly increased with 1 mmol/L excess phosphate present. This study was to determine the solubility of HA over the pH range 3.3–5.4 with a series of concentrations of such 'excess' phosphate with solid titration (ST), and to identify the precipitate formed at equilibrium with SEM, TEM, EDX, FTIR and XRD. For $[PO_4]_{XS}=0–0.3$ mmol/L, results followed closely the already-reported ST 'low' solubility isotherm. At $[PO_4]_{XS}=0.5$ mmol/L, the solubility surface switched abruptly to a 'high' position that could not be reconciled with either the 'low' isotherm or conventional calculations. Thus, HA solubility is critically dependent on the presence of excess phosphate. Such excess may account for the discrepancy between ST and excess-solid results, although the crystallographic explanation is as yet lacking.

Keywords: Biomaterials; Solubility; Solid titration; Phosphates

1. Introduction

The archetype of the apatite mineral family, hydroxyapatite (HA) is of fundamental importance in a variety of fields, such as soil science, purification processes, medicine, and biological materials. Its solubility has been much studied over several decades due to its importance as a model for biological hard tissue, but in particular in the context of dental caries, fluoridation and remineralization. However, results have varied markedly from one report to another. But whether as a result of differences in methods, raw materials, test solutions, or other factors, a definitive statement of its true solubility is still lacking. This can be ascribed to the rather complicated dissolution process of calcium phosphates in general, and even for well-characterized synthetic pure HA, despite considerable effort over a long period [1–10].

The conventional approach to the determination of HA solubility uses a relatively large amount of solid immersed in an aqueous medium for between a few hours and a few days. When equilibrium is assumed to have been reached, the pH is noted and the mass or the solution concentration of calcium is checked so as to determine solubility [6,11–14]. However, superficial phase transformation leading to a lower Ca/P ratio can occur, and this has often been ignored. The apparent solubility cannot then reflect the true behavior HA due to this incongruent dissolution [2]–the solution composition does not match that of the solid in contact. Incongruent dissolution invalidates all such 'solubilities' because the value depends on the amount of solid present, in violation of the underlying thermodynamic assumption.

The dissolution mechanisms of apatite are thought to be extremely complex, involving several simultaneous aqueous and surface reactions, so that a single solubility product could not govern the whole process if it involves several solid phases. Consequently, a surface complex has been proposed to explain the phenomenon [15,16]. However, the exact identity of such a material is controversial due to difficulties with the

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0272-8842/$ - see front matter
http://dx.doi.org/10.1016/j.ceramint.2013.10.044

model and the poor sensitivity of the test methods, there being no direct evidence of such a material.

Given all this, and to avoid transformations, Leung and Darvell developed solid titration (ST) [17], since confirmed as reliable and precise in determining the solubility of HA and related substances under closely-defined conditions [3,18–23]. It is a key finding that the solubility of HA as determined by ST is substantially lower than commonly reported [3,20]. In addition, the equilibrium precipitate has shown no sign of a special coating or complex, although its Ca/P ratio decreases slowly with lowering equilibrium pH [20]; this has also been observed elsewhere [24,25].

The effect of other solution ions (*i.e.*, through speciation effects) has not drawn much attention, or at least its importance has been underestimated. Although interaction between HA and ions such as K^+, Na^+, and Cl^- can ordinarily be neglected [2], the effect of 'extra' (analytical) background [Ca] and $[PO_4]$, and ions such as Sr^{2+}, F^- and CO_3^{2-} should be considered. For example, it has previously been shown that the solubility of HA is significantly increased by solution carbonate and phosphate [3]. Although it is a different mechanism, ions incorporated in the apatite lattice, where the substitution may cause crystal lattice distortions, result in changes in physicochemical properties, including solubility [19,23]. Indeed, it has been shown that the solubility of bovine bone mineral, a carbonated apatite, is significantly higher than that of pure HA [18]. Likewise, solubility in a simulated saliva is substantially higher than in 100 mmol/L KCl, with or without CO_2 present [3,26]. Hence, any attempt to investigate the behavior of HA by immersing the solid in a simulated body fluid or buffer solution, which necessarily contains various ions whose roles have not been investigated, should be considered with caution [11,13,14,27]. Similarly, but with the greater force, the use of phosphoric acid as a pH regulator or test background solution [28–32] is incorrect, or unsuitable at the very least, in the absence of a proper recognition and characterization of its effects.

A preliminary observation of the effect of phosphate on HA solubility was of slight increase for pH 4.2 to 5.0, with 1 mmol/L excess phosphate [3]. However, it is clear from the above that more detailed investigation is required. Hence, the aim now was to determine the solubility of HA in the presence of a range of background $[PO_4]$ concentrations, and to identify the equilibrium solid.

2. Materials and methods

Solid titration was used as previously described [3,18–23] to determine the solubility of HA in the test solution at 37.0 ± 0.1 °C. In essence, the method depends on determining, by a laser-scattering detector, the point at which no further solid may dissolve, or at which a new precipitate forms, using small increments of solid that must dissolve completely before a further increment is added.

Pure HA, prepared by a standard precipitation method in a closed borosilicate glass reaction vessel flushed with N_2, was the titrant solid. High-purity KCl and KH_2PO_4 (ARISTAR, BDH, Poole, England) were used to prepare the test solutions with ultra-pure deionized water (arium 631; Sartorius, Goettingen, Germany). All solutions contained 100 mmol/L KCl, in which was included 0, 0.1, 0.2, 0.3, 0.5, 1, 2, 10, 50, 100, or 500 mmol/L KH_2PO_4 (*i.e.*, to provide 'excess' phosphate, $[PO_4]_{XS}$, where the form $[PO_4]$ refers to analytical phosphate, without regard to speciation), the pH being adjusted as required with 5 mmol/L HCl or KOH solution. The reaction vessel and its contents were purged slowly and continuously with water-saturated nitrogen at 37 °C through an immersed capillary to eliminate and exclude CO_2 [20]. The vessel contents were stirred with a magnetic bar at ~60 rpm, sufficient to keep solid particles suspended. Runs were interlaced, randomized in groups, and many replicated on several distinct occasions over three years, to ensure repeatability.

The solid titration process entailed the following. When thermal (± 0.01 K) and pH (± 0.001) stability was attained for 600 mL of the test medium, and the scattering detector output on a chart recorder seen to be stable, the first increment of solid was added. Dissolution gave a quasi-exponential decline in scattering signal after the initial abrupt rise. When the scattering signal had returned to baseline, as near as could be judged, the system was allowed to equilibrate for at least a similar interval to ensure dissolution of the increment. The next and subsequent increments were treated similarly, until the scattering signal remained elevated with no indication of decline over several hours. Having established that the end-point had been exceeded, a further small increment was added such that after equilibration approximate interpolation for both solid added and end-point pH could be made using the scattering signal as a guide [3]. When this process was complete, a quantity of 5 mmol/L HCl was added, enough to dissolve completely the solid and enable the commencement of the next run from a new initial pH, eventually covering the pH range ~3.3–5.4. Above about that limit, the solubility is too low to be determined reliably. Likewise, at low pH, the solubility becomes rather high and more difficult to determine with ST.

To obtain a sufficient quantity of end-point, equilibrated precipitate for characterization, the ST was extended on selected occasions by continuing the incremental addition to a total of a further 6–10 mg, as described in detail elsewhere [20] (it has been previously been shown that all such added material also dissolves to be reprecipitated–no remnants are detectable). This was done for end-points both above and below pH 3.9 where the break in the continuity of the isotherm occurs [20]. After a further 10 days' equilibration, precipitates were collected by centrifugation (2000*g*, 15 min), rinsed quickly by several droplets of ultra-pure deionized water (to reduce the amount of KCl and KH_2PO_4 carried over; this had been found not to have any detectable effect on the precipitate), and dried in air at 60 °C overnight [18].

The ST here was in three stages. Firstly, using HA in 100 mmol/L KCl only, to reconfirm the reproducibility of the standard ST isotherm for HA [3,20] with the current set-up, materials and operator. Secondly, solubility was determined for the series of excess phosphate concentrations, $[PO_4]_{XS}$. Equilibrated precipitates were collected for $[PO_4]_{XS}=0.5$ mmol/L. Thirdly, HA was rerun for $[PO_4]_{XS}=0$ and various other values, several times each, to recheck reproducibility. The response surface (*i.e.*, the three-dimensional behavior plot) was fitted by Loess smoothing (degree 2, 30% data proportion, no exclusion of outliers) (SigmaPlot v9, SPSS, Chicago, IL, USA) [33].

Titrant and equilibrated precipitate morphology was observed by scanning (SEM) (Quanta 400F, FEI/OXFORD/HKL, Eindhoven, Netherlands) and transmission (TEM) (JEM-2010HR, JEOL, Tokyo, Japan) electron microscopy. Ca/P ratio was determined by elemental analysis using energy-dispersive X-ray analysis (EDX) (Quanta 400F). Constitution was characterized by X-ray diffraction (XRD) (D/max 2200 *v*, Rigaku, Tokyo, Japan) using Cu Kα radiation in step-scan mode ($2\theta=0.02^\circ$ per step). Phase identification was by comparison with the standard HA powder diffraction file (PDF2-2004; 09-0432) [34]. Functional groups were identified by Fourier-transform infrared spectroscopy (FTIR) (Vector 33, Bruker, Ettlingen, Germany) between 2000 and 400 cm^{-1} with a resolution of 2 cm^{-1} and using 100 scans.

For comparison, solubility isotherms for HA at various pH values over a range of Ca/P ratios, using a comprehensive speciation [17], were calculated in RAMESES [35–38] using $pK_{ip}=58$ as a representative value. A response surface was fitted as above.

3. Results

3.1. Characterization of HA and precipitates

Titrant HA was well crystallized, with a crystal size of 50–100 nm (Fig. 1) and Ca/P ratio = 1.68 (Table 1). The crystallites, however, were found in sintered clusters, with clear fusion interfaces. The discrepancy from the stoichiometric value (1.67) is within detection error limits. The XRD pattern matched well the standard card data (PDF2-2004; 09-0432) [34], indicating good crystallization, with no other phase detected (Fig. 2). No carbonate was detected in the titrant HA (Fig. 3).

In respect of morphology (Fig. 1), composition (Table 1), XRD pattern (Fig. 2) and FTIR spectrum (Fig. 3), the precipitates formed at pH ~3.8 and ~4.3 for $[PO_4]_{XS}=0.5$ mmol/L were similar to the titrant, although the Ca/P ratio was lower (consistent with previous work [21]) and the crystal size (25–50 nm) was smaller, but as clearly separate crystallites. These patterns are not distinguishable from those previously

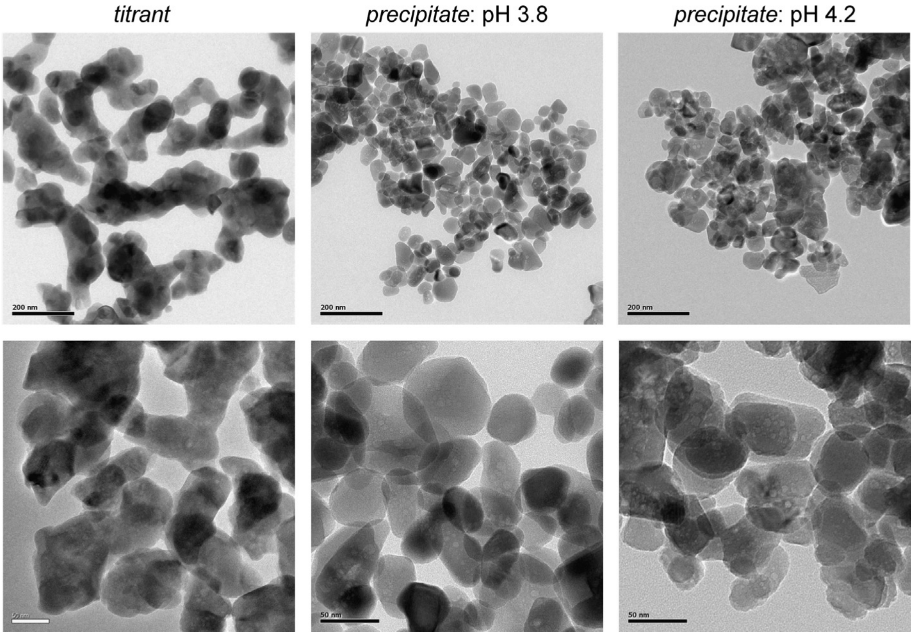

Fig. 1. TEM images for the precipitates formed at pH 3.8 and pH 4.2 for $[PO_4]_{XS}=0.5$ mmol/L compared with that of the titrant HA.

Table 1
Composition of titrant, and precipitates collected for $[PO_4]_{XS}=0.5$ mmol/L, determined by EDX.

Material	Elemental composition/at%								Ca/ P
	Ca	P	O	Na	Mg	C	K	Cl	
Titrant HA	24.46	14.51	60.88	0.00	0.15	0.00	0.00	0.00	1.68
Precipitate at pH 3.8	21.44	16.32	62.24	0.00	0.00	0.00	0.00	0.00	1.31
Precipitate at pH 4.3	23.73	15.01	61.26	0.00	0.00	0.00	0.00	0.00	1.58

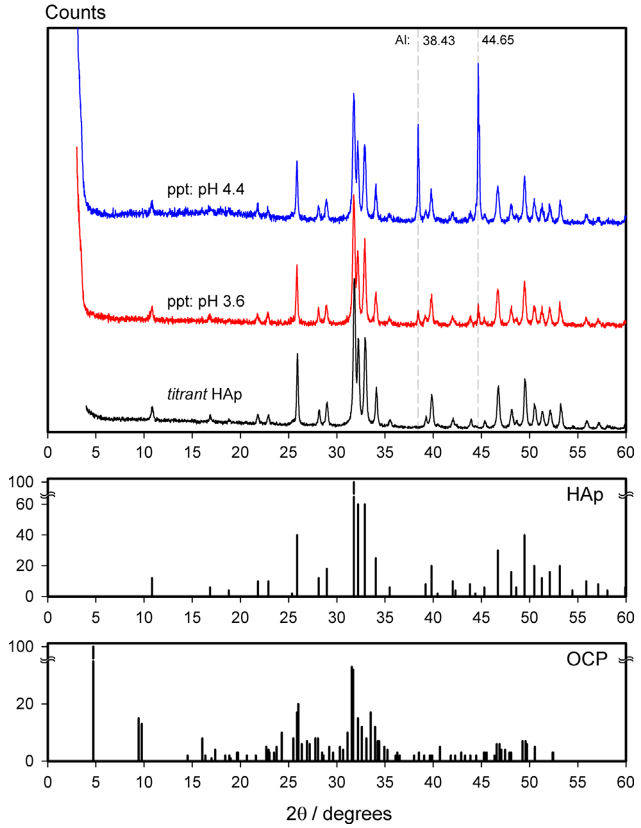

Fig. 2. XRD patterns for the precipitates formed at pH 3.6 and pH 4.4 for $[PO_4]_{XS} = 0.5$ mmol/L, compared with that of the titrant HAp. Note the absence of any indication of the presence of OCP (in particular, at $2\theta = 4.7°$). Spurious peaks are present in the precipitate patterns attributable to the aluminium of the stub showing through because of the small amounts available.

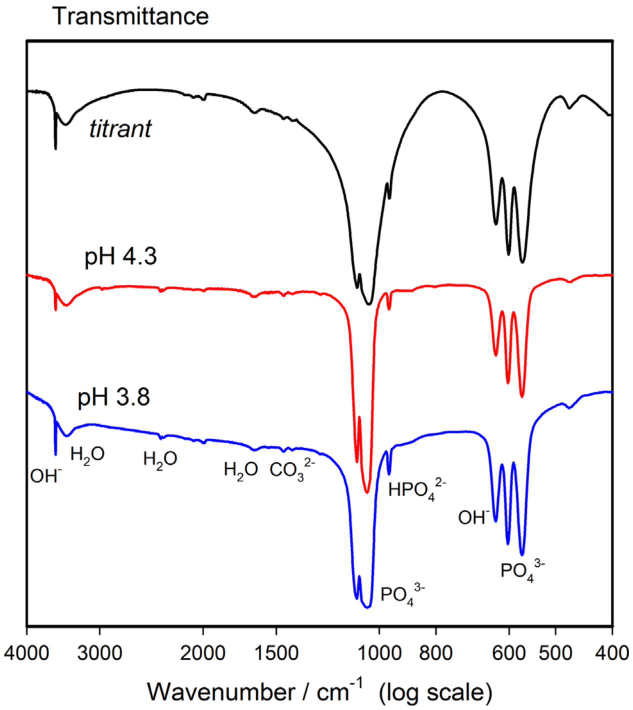

Fig. 3. FTIR spectra for the precipitates formed at pH 3.8 and pH 4.3 for $[PO_4]_{XS} = 0.5$ mmol/L compared with that of the titrant HA.

obtained for $[PO_4]_{XS} = 0$ [20]. Again, carbonate was not found (Fig. 3), and in particular there was no indication of the presence of octacalcium phosphate (OCP) (Fig. 2).

3.2. Solubility of HA

The titration end-points are shown in Fig. 4a vs. log(Ca/P), while the coverage of the titration in terms of pH and Ca/P ratio is shown Fig. 4b: there are 101 points from previous work [39] and 12 new (system verification) points at $[PO_4]_{XS} = 0$. There are 333 new points (this study) for $[PO_4]_{XS} > 0$; plus 12 points from a previous study [3] for $[PO_4]_{XS} = 1$ mmol/L. A view of the three-dimensional plot of all data is given in Fig. 4c. The results fall into two domains: for $[PO_4]_{XS} \geq 0.5$ mmol/L–"high XS", and $[PO_4]_{XS} = 0$–0.3 mmol/L – "low XS", which are distinguished by the latter being clearly not smoothly-continuous and consistent with the locus of the high XS points. The response surface (Fig. 4d) was fitted on this basis, avoiding an abrupt, essentially discontinuous, reversal of slope that would make no sense chemically in terms of solubility alone. The old data for $[PO_4]_{XS} = 1$ mmol/L are consistent with the new data and fall on the same response surface, while the data from the various runs at a given value of $[PO_4]_{XS}$ were not distinguishable. The exclusion of some points for $[PO_4]_{XS} = 500$ mmol/L for the surface fitting was on a slightly different basis. While the plot for these values in Fig. 4a appears consistent, in Fig. 4b they have a distinct V-shaped path not found for any other set, suggesting that some other process was now involved or that the domain of behavior was now different. Thus, the seven points running from ~pH 3.45 to 4.2, at $\log(Ca/P) < -3.8$, introduced a strong but local distortion to the surface (*i.e.* at very low Ca/P) and so were excluded from the fit. Even so, there was no appreciable effect at and near the high Ca/P boundary if included.

4. Discussion

In order to understand ST results, it is necessary briefly to rehearse some key points. While it is understood that the solubility of HA, as for all solids, varies with precise composition, crystallite size and perfection, as well as phase purity [8,12,23,40,41], none of these is of any concern here since it is only the newly-precipitated material that is of interest, and all titrant is always dissolved. This can be ascertained from SEM inspection: the sintered crystallites are entirely missing from the recovered precipitates, whose crystallites are uniformly separate particles (Fig. 1). Factors that might inhibit dissolution and thereby cause apparently anomalous results cannot pertain. The precipitate is necessarily close to an equilibrium state [20], especially after the extended incubation it is given, and obviously could not form in the first place unless there was some slight supersaturation, that is, formation from an undersaturated solution is not possible, of course. The crystallite size effect may mean that the prepared precipitate becomes more homogeneous in particle size range on equilibration, and necessarily larger with time. This means that the initial end-point, with the smallest detectable particles, lies very slightly above the true isotherm, solubility decreasing (very slightly) on ageing–Ostwald ripening. In addition, ST avoids the

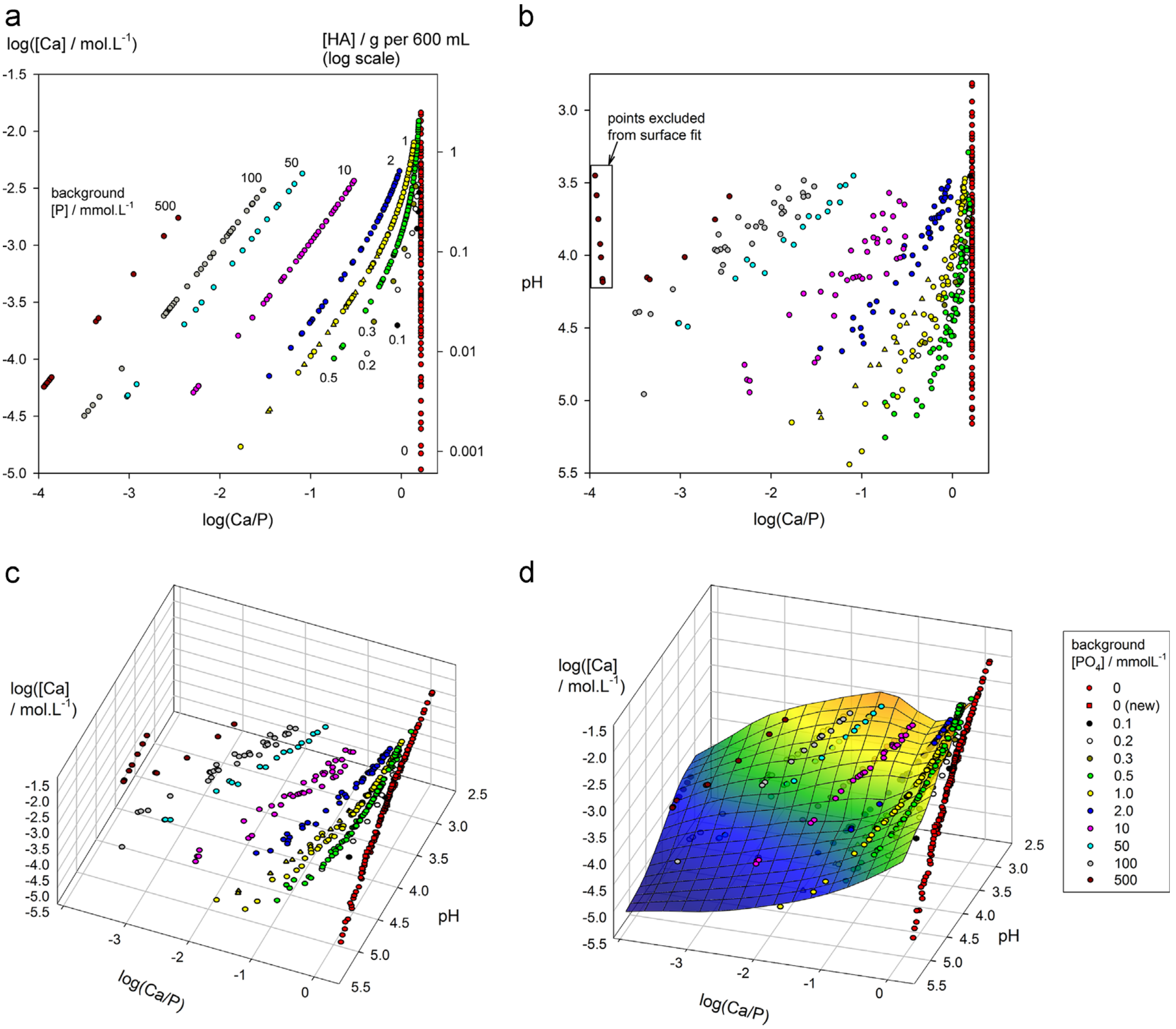

Fig. 4. Solid titration results in 100 mmol/L KCl at 37 °C. (a) Range of end-point calcium concentrations vs. pH, and (b) the pH scope vs. range of Ca/P values, (c) three-dimensional overview, (d) response surface fitted to the data of (c) including values for $[PO_4]_{XS} \geq 0.5$ mmol/L, but excluding those points marked in (a) and (b) by boxes for $[PO_4]_{XS} = 500$ mmol/L. [Ca] determined from total titrant added, $[PO_4]$ from titrant plus $[PO_4]_{XS}$. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

complication of incongruent dissolution since the solution composition at the end-point is known accurately (by interpolation [3,18–23]), corresponding to the total titrant added. In the case of $[PO_4]_{XS}=0$, the Ca/P ratio is always precisely that of the titrant. The method is absolute for the same reason: the solution composition at the end point corresponds very closely to the total, known, added titrant (*i.e.* negligible precipitated solid, of the order of 1 mg, not visually-detectable). There are no assumptions for calculations beyond the errors of weighing; in particular, there is no reliance on compositional analyses for end-point determination or calculated equilibria.

Spurious variation from procedural errors or equipment conditions is discounted since the consolidated $[PO_4]_{XS}=0$ results of several previous operators, at two sites ("old" data, Fig. 5) [3,20], have been reproduced (the 12 "new" points conform closely to the track of the "old" data, indistinguishably), indicating that the experimental system behaved consistently. These data together will, for convenience, be referred to as the HA_0 results.

The ordinary implication of excess in solution of a component of a solid is to lower the apparent solubility by mass action, a simple result of the ion product-driven behavior. Evidently, for raised solubility to be observed other factors must be involved.

It is apparent (Fig. 4d) that the presence of excess phosphate has a dramatic and discontinuous effect on the apparent solubility of HA. The possibility of an error or a noise effect can be discounted as the discrepancy at the high-Ca/P edge is much larger than the scatter. The fact, then, that the response surface for $[PO_4]_{XS} \geq 0.5$ mmol/L does not sensibly contain the isotherm for HA_0 seems to lead immediately to several deductions:

(1) Excess phosphate is critical in causing a switch to a substantially different behavior, most notably at $[PO_4]_{XS}=0.5$ mmol/L. For comparison with the total calcium present, note that $\log(0.5 \times 10^{-3}) = \sim -3.3$, *i.e.* rather low compared with that over most of the range covered, indicating considerable

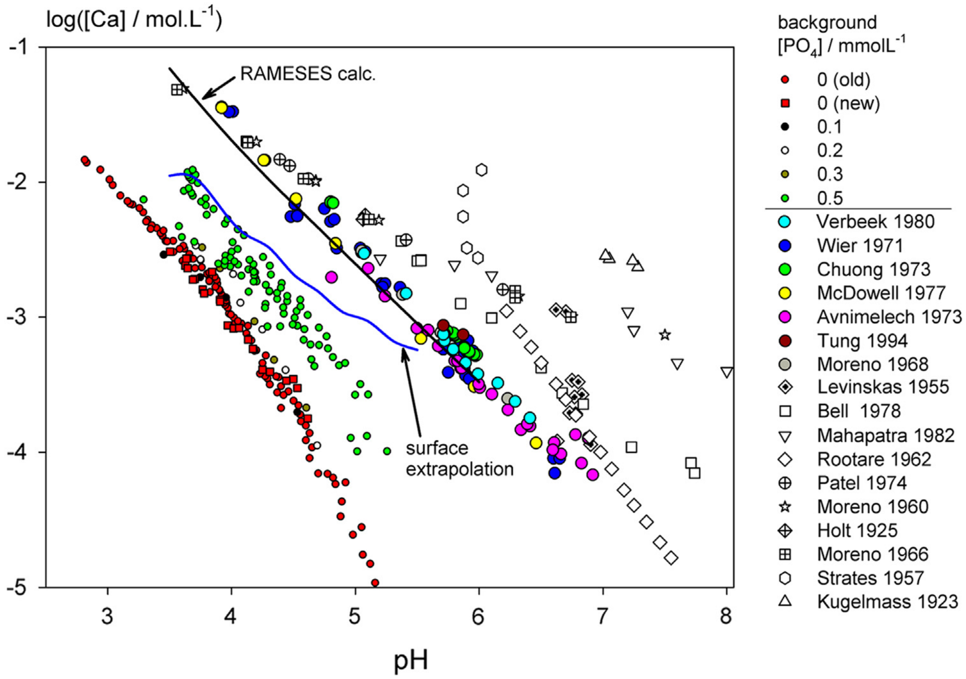

Fig. 5. Comparison of HA_0 results with those for $[PO_4]_{XS}=0.1$–0.5 mmol/L, intersection of extrapolated response surface of Fig. 4d with Ca/P = 1.67 (wavy line), calculated solubility isotherm based on $pK_{ip}=58$ (smooth line), 'excess solid' results that correspond to that calculated isotherm (*i.e.* the data that underlie that pK_{ip} value) (large filled circles), and 'excess solid' results that are broadly discrepant (large open symbols). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

sensitivity. Even so, the results for $[PO_4]_{XS}=0.1$–0.3 mmol/L indicate a limit to that sensitivity in that these are not associated with the high XS response surface (Fig. 4d). Evidently, there is a threshold which needs to be exceeded, but it has, nevertheless, a rather low value. Curiously, the threshold is apparently not appreciably pH- or dissolved-"HA" concentration-sensitive as the remark holds good over the range covered. This unexpected behavior was checked and rechecked several times with additional independent runs (all included in the data set now).

(2) The fact that the pH locus of the smoothed surface lies well above the line of the HA_0 results for $Ca/P \to 1.67$ implies the likelihood of a different phase over the whole pH range. That is, if it is legitimate to suppose that the magnitude of the discrepancy, and the slope of the fitted surface, along the high-Ca/P ratio edge can be taken as evidence that this surface is not continuous with the HA_0 data. However, there is no indication of any other phase in the precipitates, and especially not of OCP, which has been suggested to be a significant risk in such systems (R.G. Hill, *pers. comm.*)

(3) This phase–whatever it is–must be, by definition, less stable than the HA_0 equilibrium phase, firstly because HAp is commonly understood to be the most stable of the system, and secondly the solubility now is higher, at least at $[PO_4]_{XS}$ ~0.5 mmol/L.

(4) This in turn implies that it is more easily nucleated under the conditions used than the HA_0 equilibrium phase. This is a common behavior in the sense of an Ostwald succession when approaching 'from above', as it were. However, here the approach is 'from below' by successive additions of small increments. Precipitates otherwise normally appear promptly at $[PO_4]_{XS}=0$, and indeed this has occurred in all other relevant titrations, nucleation by the titrant probably being responsible, as would be expected.

(5) It then follows that this 'high' phase is necessarily metastable with respect to the 'low' HA_0 phase.

(6) Even so, conversion clearly does not easily occur since several days of incubation were always used to establish the equilibrium, and massive reprecipitation never occurred (*cf.* the behavior described for DCPD titration and the effect of seeding [21], and likewise for OCP titration and seeding [42]). It should be noted that the method used to establish the end-point always involved at least two steps beyond the first indication of precipitation so that interpolation for improved end-point identification could be used [21]. It would be supposed that this (at least temporary) coexistence, in at least some instances, of titrant and 'high' precipitate would cause seeding of the conversion, and a correspondingly large amount of material coming out of solution, noting as well that the titrant HA appears to be a perfectly good seed otherwise. Thus, the 'low' phase does not nucleate on the 'high', which may parallel the failure of DCPD to nucleate HA [21].

(7) Thus, the effect of the excess phosphate appears to be to block nucleation of the 'low' phase, and in particular on titrant added beyond the HA_0 expectation (which is then, in effect, 'excess solid'), but that titrant nevertheless continues to dissolve until the new 'high' endpoint is reached.

This extraordinary, seemingly paradoxical conclusion can be examined in the light of two matters: firstly, previous studies of HA solubility in the presence of excess phosphate and using the excess-solid method; secondly, the ordinary expectations of solution chemistry.

The behavior for $[PO_4]_{XS}=0.5$ mmol/L is brought out by comparison with the data for $[PO_4]_{XS}=0$–0.3 mmol/L (Fig. 5). While some points at lower pH do not appear to be particularly discrepant, the majority clearly are, albeit with some scatter. Extrapolating the high XS fitted surface to Ca/P = 1.67 yields the wavy line as the implied behavior at $[PO_4]_{XS}=0$. Obviously, the position of this is subject to appreciable uncertainty (and its waviness an artefact of the surface fitting), but nevertheless it makes the point well enough: there is a general minimum difference of a factor of 3 above the 'low' HA_0 isotherm, *i.e.* for $[PO_4]_{XS}=0$.

It is instructive to compare the results of previous solubility determinations (Fig. 5). Taking $pK_{ip}=58$ as a representative value for the conventional solubility of HA (as $Ca_5(PO_4)_3OH$), we may compare those results with the implied solubility isotherm (black line). It is immediately apparent that relatively few of those other studies are at all consistent, with a large number of determinations (white symbols) showing large discrepancies and, as has been shown before, these cannot be accommodated by invoking Ca/P variations alone [39]. However, as has also been pointed out [39,43], the calculated solubility in such a system depends critically on the solution speciation model–complexes and ion pairs–and a simplistic approach is doomed. (It is necessary to recall that all results from excess-solid work depend on calculations from measured solution concentrations and a series of assumptions, unlike the absolute determination by ST of a physical endpoint.) Accordingly, calculations need to take this into account, although this is not often done [39]. Thus, isotherms as shown in Fig. 6 (heavy *J*-shaped curves) are obtained when the model is more complete. The corresponding fitted surface is shown to be distinct from that fitted to the present experimental results by virtue of both its shape and separation: a simple scaling shift (*i.e.* adjusting the value for pK_{ip}) could not bring them into coincidence, even approximately.

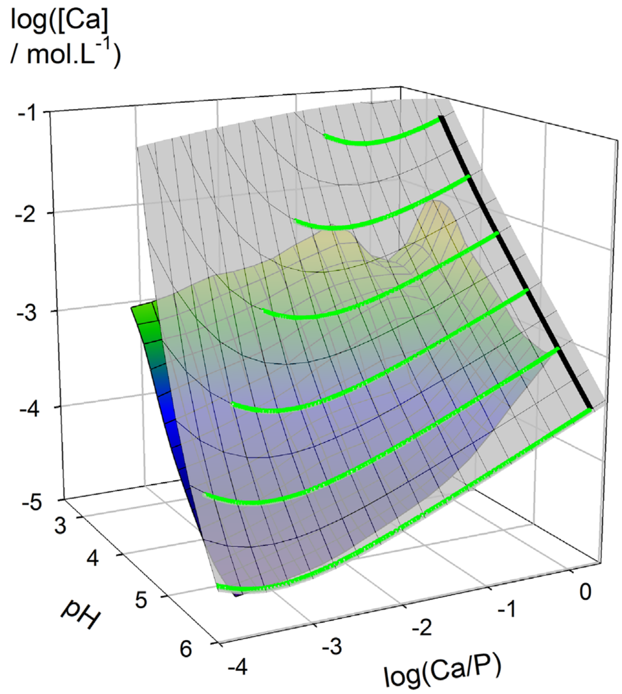

Fig. 6. Solubility of HA in 100 mmol/L KCl at 37 °C vs. pH and log(Ca/P) ratio. Comparison of the present experimental results fitted surface (Fig. 4d) with that fitted to calculated values from scans in Ca/P value (heavy J-curves) (the solid straight line corresponds to the calculated isotherm of Fig. 5).

In Fig. 6, then, it is clear that the experimental response surface now is rather more complex than the ordinary model implies. The general mass-action effect is present, but only with respect to the implied isotherm at $[PO_4]_{XS}=0.5$ mmol/L. Even so, the predicted reversal to greater solubility has no counterpart in the experimental data surface except in a restricted region at low pH and high Ca/P ratio, and then in a much sharper fashion. Some exploration of modified equilibrium constants and new solution species (such as $Ca(HPO_4)(H_2PO_4)^-$ and $Ca(H_2PO_4)OH$ [39]) failed to indicate how such a shape might arise. In any case, no such modification could explain the abrupt switch to the "high" surface at only $[PO_4]_{XS}=0.5$ mmol/L.

As has been shown before [3,20], the ST results for HA_0 are distinct from conventional excess-solid results in that one branch of the experimental isotherm (at pH > ~3.9) cannot be explained by a simple modification of the value of the 'normal' HA pK_{ip}–it has the wrong slope. Again, this cannot be made to conform by any simple modification of the calculation: extensive exploration of the implications for the solubility curves for solids over a wide range of Ca: H: PO_4: OH combinations failed to provide any clue.

Numerous studies, over many years, have been made in an attempt to understand the dissolution behavior of apatite, yet disagreement persists, a problem ascribed to limitations in the methods, specimen differences, and the investigative models [1–4,6,8,9,11–14,20,27,28,30–32,40,44,45]. All methods supposedly based on thermodynamic theory seem to fail, given the incongruent dissolution of HA (in which calcium is preferentially dissolved [14]) and the lack of an adequate solution speciation model [46]. In any case, phase transformations in the excess-solid method are unavoidable, and these have never been dealt with satisfactorily. As is apparent from data compilations [3], there is no consistency, and the expectation is that they deviate appreciably from the true values. In contrast, ST was designed specifically to avoid the confounding of results by such phase transformations [17]. The difference between ST results and those other values is stark [3,20]. In particular, no coating or second phase has been found in the equilibrated precipitate [20].

Strangely, phosphoric acid has often been used for pH adjustment in HA solubility studies, or even as the test background solution, ostensibly to minimize contamination by other ions [28–32,47–49]. However, given the present results, the use of phosphate in this way is suspect, to say the least, and the results of such work must be treated with caution. According to ordinary mass-action expectations, phosphate already in solution should suppress dissolution of HA and thus yield a depressed

Table 2
Key to labels of Fig. 7. Numbered species are from Table in [54], X is from PDF2-2004: 46-0905 [55], Y from [56].

No.	Formula	Abbrev.	
23	H_3PO_4		
25	H_3PO_4. $0.5H_2O$		
26	H_3PO_4. H_2O		
27	$Ca_3(PO_4)_2$.CaO	TTCP	tetracalcium phosphate
28	$Ca_3(PO_4)_2$	TCP	tricalcium phosphate
29	$Ca_3(PO_4)_2$. $0.5H_2O$		
30	$Ca_3(PO_4)_2$. $3H_2O$	TCPT	tricalcium phosphate trihydrate
31	$Ca_9(PO_4)_6$. $2H_2O$		
32	$3Ca_3(PO_4)_2$. $Ca(OH)_2$	HA	hydroxyapatite
33	$2Ca_3(PO_4)_2$. $Ca(OH)_2$		
34	$Ca_3(PO_4)_2$. $Ca(OH)_2$		
48	$CaHPO_4$	DCPA	dicalcium phosphate, anhydrous
49	$CaHPO_4$. $0.5H_2O$		
50	$CaHPO_4$. H_2O		
51	$CaHPO_4$. $1.5H_2O$		
52	$CaHPO_4$. $2H_2O$	DCPD	dicalcium phosphate dihydrate
54	$Ca(H_2PO_4)_2$	MCP	monocalcium phosphate
55	$Ca(H_2PO_4)_2$. H_2O	MCPM	monocalcium phosphate monohydrate
60	$Ca_8H_2(PO_4)_6$ $0.5H_2O$	OCP	octacalcium phosphate
63	$Ca_8H_2(PO_4)_6$	OCPA	octacalcium phosphate, anhydrous
69	$Ca_2H_2(PO_4)_2$. $2H_2O$		
70	$Ca_5H_2(PO_4)_4$. $4H_2O$		
X	$Ca_9(PO_4)_6$ .H_2O		calcium hydrogen phosphate hydroxide
Y	Ca_2PO_4OH . $2H_2O$		isoclasite

solubility isotherm. However, the actual behavior is counter to that expectation, up to at least $[PO_4]_{XS}$=10 mmol/L. In the 1960s, “acidulated phosphate fluoride” (APF) was introduced as a means of getting more fluoride deposition on teeth with (supposedly) minimal demineralization, on the claimed basis that by mass action the solution phosphate (at 100 mmol/L), at pH 3–4, limits the dissolution of enamel during the treatment [50]. However, the data in that paper show that dissolution actually increases with $[PO_4]$ (their Tables 1 and 2). Perhaps the chemistry of the rationale needs to be reconsidered, but in the present sense the role of phosphate is not as would ordinarily be expected, lending some indirect corroboration to the present observations.

The results that have been obtained using solid titration admit of no simple explanation, as has been explored previously [3,19,20], a fact that continues to exercise us (and many of our colleagues, to say nothing of a succession of reviewers). Various possible causes have been mooted and all discounted [3,19,20]. Likewise, we have no satisfactory explanation for the observations here regarding the effect of $[PO_4]_{XS}$, and systematically we have eliminated potential problems raised before. One factor that has been suggested informally to be involved is magnesium, on the grounds that it inhibits HA nucleation [51]. This, if anything, would tend to bias the ST endpoint upwards, *i.e.* raising the apparent solubility. However, the concentration required to exhibit such an effect appears to be rather greater than could possibly obtain here. The materials used in this work were all AR or better (KCl: $<0.0001\%$; calcium nitrate tetrahydrate $<0.01\%$, di-ammonium hydrogen phosphate: none stated). Attempts to measure [Mg] in the titrant and products using EDX produces maximum values of the order of 0.15 mass%, which are barely acceptable analytically (it is hardly distinguishable from the background noise), but inexplicable in terms of source materials and handling. But even if this represents the amount present accurately, in the very worst case, *i.e.* at pH 3 and high Ca/P ratio, the maximum possible concentration in the solution at the endpoint would be 0.3 mmol/L (given that the titrant added was then 2.5 g/L; Fig. 4a); over most of the range covered it would be 1–3 orders of magnitude smaller. Nevertheless, even such a high upper-limit figure is substantially below the level at which an effect has been detected elsewhere, *i.e.* 1 mmol/L [Mg] [51]. However, end-point detection in ST is always prompt. That is to say that one does not have to wait long (of the order of a few minutes only) for end-point precipitation to be detectable after an increment has been added. Even then, the amount of precipitate after equilibration is very small, meaning that the degree of supersaturation is always very small, both as intended. If appreciable inhibition were involved, such that the degree of supersaturation required were high (bearing in mind that each increment always provides nucleation sites), then a large precipitation would be found (as normally occurs in metastable solutions) after appreciable overshoot. This has never been seen for HA titration, although something similar occurred for DCPD ST [21]. Nevertheless, the effect of Mg in HA is to raise the solubility [52–54] and incorporation does not occur even in Ca-deficient HA in 100 mM $MgCl_2$ [51]. Hence, what is precipitated at the end-point is necessarily much lower in [Mg] than could be in the titrant (that is, if it is present, it must

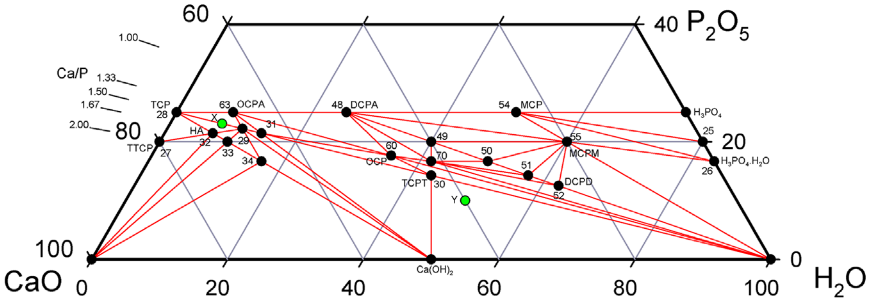

Fig. 7. Portion of the triangulation diagram of Aldabergenov and Balakaeva [54]. The key is given in Table 2. Ca/P ratios are indicated on the left, the isopleths passing through the H_2O apex. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

be partioned preferentially into the solution, reinforcing the view that the EDX results are unhelpful), and thus of lower solubility anyway, even if Mg acted to inhibit at such a concentration (for which we have no evidence). Again, in ST all titrant must dissolve (as confirmed by TEM inspection of precipitates, Fig. 1), and the solubility isotherm drawn cannot in fact be raised by any such contamination: delayed or inhibited dissolution must yield a low value, not a high. The reproducibility of the $[PO_4]_{XS}=0$ results (interlaced in time as they were here now with the $[PO_4]_{XS}>0$ results) with previously obtained data speaks of no local or special conditions underlying the present outcome for the effect of $[PO_4]_{XS}$. It is also worth noting that we are aware of no instance of work such as those cited here in which special care was taken to eliminate Mg from the materials of HA or Ca-P solubility studies (or the risk even mentioned for this or other potential contaminants), and that were there to be such a problem it must apply to all such excess-solid work, where the partitioned concentration [Mg] must necessarily be much higher than in an ST system (because of the large excess of solid used) and therefore *a fortiori* be a bigger problem. Even so, it is planned to make an explicit test of the effect of solution magnesium as part of the program of work on this subject. Meanwhile, we are not convinced that it is likely to account for any aspect of the ST results now, although we continue in our search for an explanation, even if it is a fatal flaw in the method–which in itself would be revealing of the chemistry of the system (and none has yet been identified by any critical observer). The lack of an explanation to offer now cannot logically undermine the observations: something is causing them.

There would appear to be only two main mechanisms for the results now obtained. Firstly, the crystallographic detail of the solid, and secondly, solution speciation. The solid may exhibit a phase change due to ordering (perhaps some kind superlattice), complicated by protonation of the phosphate, vacancies (especially of calcium, of course), or included water. In this, location of the hydrogens would seem to be key (neutron diffraction?). Turning to solution speciation, this is normally parsimonious: the minimum number of postulated equilibria to fit the data (of whatever kind), but high noise in such data tends to make crude approximations adequate. It could be that a number of other species need to be invoked, but unless other equilbrium constants are adjusted 'downward', mere addition can only increase the complexation titer and thus only raise solubility. An independent assay of, say, distinguishable phosphate solution species would provide valuable data, but as far as we know this cannot be done by such as NMR. Even so, discontinuity cannot result directly from such adjustments (*i.e.* as for the switch at $[PO_4]_{XS}=5$ mmol/L) unless a phase change is also involved. This may be related to the layer structure of the apatitic material, and possibly the putative octacalcium phosphate 'precursor' layer on which HA is supposed to nucleate as a twin, where the surface composition and thus surface energy of the solid is affected. We offer the above only as rather speculative and tentative stimuli to thinking about the problem.

The postulate of a phase change obviously needs to be tested by means of much more detailed investigation than has been attempted now, largely due to the minute amounts of solid that have been prepared under the conditions used here. In particular, the end-point material needs to be characterized in detail, perhaps with respect aspects of the crystallography too subtle for XRD detection now, although no candidate properties or characters have yet been identified by anyone. Nevertheless, it seems that an explanation for the present results can only lie in such a direction. Such work is now in hand.

Meanwhile, we can explore some relevant areas. The only published, low-temperature phase diagram for the system CaO–P_2O_5–H_2O seems to be that of Aldabergenov and Balakaeva [55] which, drawing on sources in Russian, was prepared on the basis of calculations of Gibbs free energies and various rules (Fig. 7). Unfortunately, in the absence of sufficient information for solution speciation, the aqueous phase was not taken into account (M. Aldabergenov, *pers. comm.*). The solidus is therefore not properly identifiable. However, what is noticeable here is the number of supposed solids in the vicinity of HA, nos. 29, 31, 33, 34, which would seem to isolate HA from stable contact with the liquid. This is supposing that such solids are soundly identified, but it is known that 'hydroxyapatite' as a structure may deviate substantially from the ideal Ca/P ratio, but also it would seem in the $[H_2O]$ fraction of the nominal formula. A parsimonious interpretation of this is of a wide range of solid solution compositions for that structure, for which there is much evidence elsewhere.

Inspection of the powder diffraction file [34] for calcium phosphates shows several cards (18-0303: $Ca_3(PO_4)_2.xH_2O$; 46-0905: $Ca_9HPO_4(PO_4)_5OH$ (see Table 2, Fig. 7: "X"); 54-0022: $Ca_{10}(PO_4)_6(OH)_2$) essentially the same as 09-0432: $Ca_5(PO_4)_3(OH)$, "hydroxylapatite", with no significant differences in pattern despite the varying Ca/P ratio, hydration and protonation. Without going into the quality of those data, it is clear that the diffraction pattern is not going to allow suitable discrimination. In addition, the claimed mineral isoclasite, Ca_2PO_4OH [56] does not sit easily in that diagram (Table 2, Fig. 7: "Y") but may well not be stable anyway, if valid.

The point here is that the chemistry of the calcium phosphates is associated with much complexity and non-stoichiometry [57] that has long been a puzzle, *e.g.* [58] and citations therein, and that this can lead to the identification of spurious species that are merely examples from continua. What is needed is the set of definitive extrema for a given crystal structure, not just the ideal formulae, in order to draw the true phase diagram in terms of solid solutions.

There remain two points. Firstly, the old results for $[PO_4]_{XS} = 1$ mmol/L, which appeared to indicate only a small elevation of solubility [3], are now seen to have been fortuitously close to the projection of the ST-derived HA_0 solubility isotherm, simply because the response surface drops so markedly (but smoothly) from the switched 'high' position with decrease of Ca/P ratio. Secondly, the excluded results for $[PO_4]_{XS} = 500$ mmol/L indicate a steeper response at very low Ca/P ratio than the surface-fitting now could accommodate. All in all, it is evident that HA solubility is much more complicated that has been hitherto suspected. Indeed, we find that there are aspects which even now appear paradoxical.

5. Conclusions

Solid titration has revealed extraordinary behavior in the solubility surface for HA in the presence of excess phosphate, which appears to control nucleation in a hitherto unrecognized fashion. Such excess may account for the discrepancy between ST and excess-solid results, although the crystallographic explanation is as yet lacking.

Acknowledgments

This work was conducted in partial fulfilment of the requirements of the degree of Master of Dental Surgery for QL at Guanghua School of Stomatology, Sun Yat-sen University, and supported by the National Natural Science Foundation of China (30973355), the Guangdong Provincial Natural Science Foundation Committee (S2011010004978) and the Sun Yat-sen University Young Teachers Fund (10ykpy16).

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