SHORT COMMUNICATION

Hydroxyapatite and Fluoroapatite Behavior with pH Change

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ABSTRACT

Background: **Due to the topical fluoride treatment, fluorapatite (FAp) and calcium fluoride are forming on a dental enamel surface. Different researches concerned in oral biology, prevention and treatment have studied hydroxyapatite (HAp) and FAp dissolution in addition to precipitation reactions since these materials are essentials in dental hard tissues. However, FAp is less soluble phase amongst the calcium phosphates and has more complicated equilibria.**

Objective: **This study has indicated the solubility isotherms, S of CaF_2 and the solubility of FAp and HAp using the titration method.**

Design: **In this study, a titration with pH adjustment method using diode laser-scattering system had been performed to monitor any solid increment addition where the material has been reprecipitated and detected by the endpoint represented by the output signal.**

Materials and Methods: **The composition and the morphology of the precipitate were studied and the Ca/P ratio was also determined. In addition, a comparative study of the solubility isotherm of the CaF_2, HAp, and FAp were performed among wax-coated apparatus and an uncoated vessel experiment.**

Results: **The results showed that Ca/P ratio of the precipitate has decreased steadily at lowering pH values. In addition, FAp has shown slightly less solubility than HAp at pH < 3.9 and the particles were single crystal with rod-like morphology at pH 4.1 and polycrystalline at pH < 3.6.**

Conclusion: **Wax-coated apparatus helped to avoid any expected interferences, the titration was successfully validated, while solutions rich with fluoride were acted as modifying agents to the solubility of HAp.**

KEY WORDS

biocorrosion, complicated equilibria, particles, phase, topical fluoride

INTRODUCTION

Fluoride has played an important role in the prevention of dental caries since the introduction of water fluoridation in the 1940s[1)], and is considered to have contributed more to the reduction in dental caries seen across the general population than any other single measure[2)].

Biocorrosion of teeth can occur as a result of exogenous chemical and endogenous biochemical acids as well as by proteolytic enzymes and piezoelectric effects on dentin[3)]. Besides, such coatings are believed to act as protective layers, and thus, to reduce acid dissolution of teeth, whether through the process of caries or from exogenously ingested acids[3)].

Recently, topical agents have been introduced which increase the amount of bioavailable calcium and phosphate, together with fluoride[4)]. Antimicrobials and xylitol are being used to reduce acidogenic bacterial loads[5)], while other modifiers have been investigated.

Two compounds - fluorapatite (FAp, $Ca_5(PO4)_3F$) and calcium fluoride (CaF2)[6)] have been detected which form on a dental enamel surface during topical fluoride treatment at various concentrations[7)]. In fact, the precipitation and dissolution reactions are occurring in suspensions of enamel, hydroxyapatite (HAp) and FAp on addition of fluoride under well-defined conditions[8)].

The solubility of FAp and CaF_2 is of fundamental importance in understanding their formation in these contexts, that is, is relevant to the biology, prevention and treatment[9)].

FAp is thought to be the least soluble phase amongst the calcium phosphates[10)]. However, as for hydroxyapatite (HAp), incongruent dissolution has been reported[11)]. If this is so, the solubility obtained by the traditional large excess of solid additions may not yield a value for the true solubility of FAp itself; phase transformations may also occur. As noted for the HAp system, the surface composition is at variance with that of the bulk in such circumstances. This is one of the most important reasons why the solubility of HAp has been found to be substantially lower by titration than the excess-solid addition technique[12,13)]. Noting that titration does not depend on equilibration with solid addition materials, but the complete dissolution of each increment. For FAp, more complicated equilibria may be involved due to the presence of fluoride. Farr and Elmore[14)] calculated a value of pKsp for FAp as 60.43 at 25℃. However, the data obtained at pH 1.76 was for mixed FAp and CaF_2, despite, the errors introduced by the large excess of solid materials need to be taken into account. A confirmation of titration results by an independent system using a numerical technique, as the RAMESES program[15)], would be of value. However, the previously noted difficulties arising from lack of detailed solution equilibria for the calculation for HAp leads to a discrepancy between experimental and theoretical isotherms. Such a gap cannot be adequately explained by known documented species. However, the simpler calcium fluoride system may be amenable for inspection by RAMESES as the solution equilibria are believed being relatively simple. Such calculated values would provide a mutual validation by concordance: verifying the reliability of the titration method as well as providing a check of the appropriateness of the

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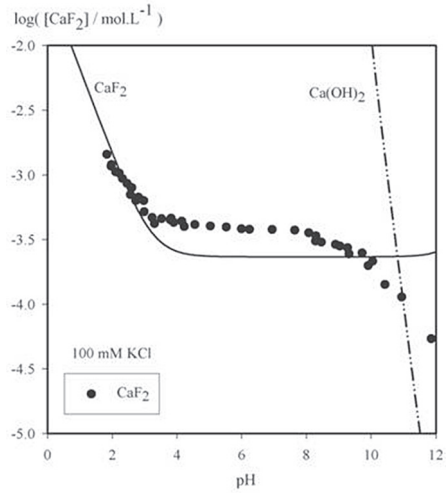

Figure 1. Experimental solubility isotherm of CaF_2 in 100 mM KCl at 37.0 ± 0.1℃ compared with the theoretical line calculated in RAMESES.

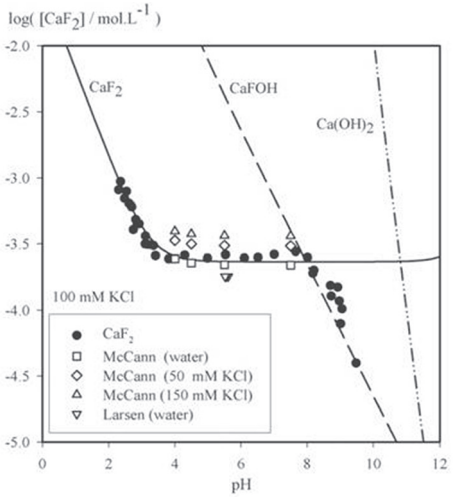

Figure 2. Experimental solubility isotherm of CaF_2 in 100 mM KCl at 37.0 ± 0.1℃, wax-coated apparatus, compared with the theoretical line calculated in RAMESES.

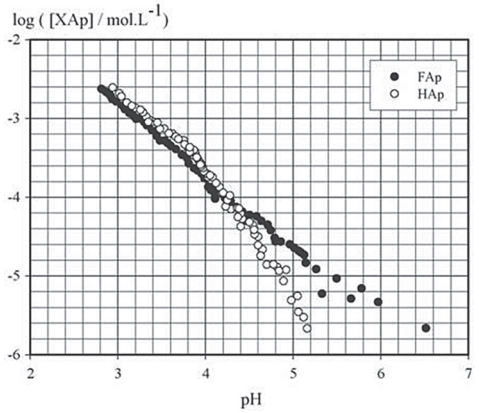

Figure 3. Experimental solubility isotherms of FAp (solid symbols) in 100 mM KCl at 37.0 ± 0.1℃. Solubility data for HAp[18] (open symbols) was used for comparison.

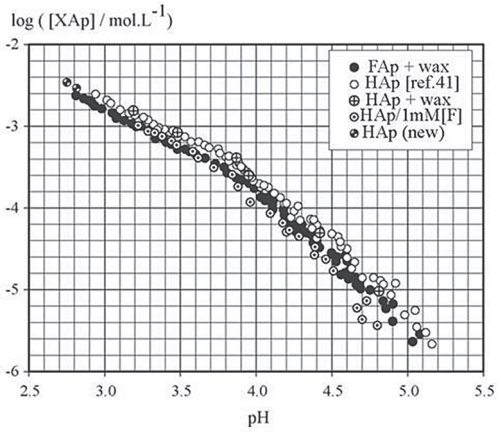

Figure 4. Experimental solubility isotherm of FAp (solid symbols) in 100 mM KCl at 37.0 ± 0.1℃, wax-coated apparatus. Solubility data for HAp[18] (open symbols) shown for comparison.

assumed speciation reactions as explanatory factors. Thus, given the importance of both FAp and CaF_2 in the context of dental hard tissue, especially with respect to topical treatment, accurate solubility data are essential. Therefore, the first aim was to determine the solubility isotherms, S of CaF_2 and FAp, with a further extension to the apparent solubility of HAp in the presence of 1 mM [F], using titration method. A second aim was a numerical check of S [CaF_2].

MATERIALS AND METHODS

Materials

Calcium fluoride (analytical grade, BDH, Poole, England) and FAp (Paffenbarger Research Centre, Gaithersburg MD, USA) were used as test materials, as well as HAp which was prepared by Sol-Gel Method[16]. For HAp test in the presence of fluoride, potassium fluoride (analytical grade, BDH, Poole, England) to 1 mM was added. Two further points at low pH for HAp alone were also determined to extend the range of comparison as well as several reproducibility check determinations.

Titration

The titration was performed in a wide-neck borosilicate reaction flask at 37.0 ± 0.1℃. The solution was flushed with nitrogen, monitored by pH electrode (Combined Electrode PHC 2002, Radiometer, Copenhagen, Denmark) and thermometer. Solid increment addition was monitored by a semiconductor-diode laser-scattering system (1mW CW, 194-010, RS Components, UK). The end-point could be unambiguously detected by the output signal being significantly higher than the original baseline. PH adjustment and redissolution were realised using hydrochloric acid. In outline, the goal is to approach saturation of the solution with respect to the components of the added material avoiding the problems caused by the difficulty of homogeneous nucleation of calcium phosphates. Substantially, solution titration overshoots the end-point. The minute amount of excess solid at the true end-point acts as its own nucleating agent, even if the added material is imperfect or a different phase from that at equilibrium. All added material must dissolve except a portion of the last increment, and then only if is exactly the stable phase and composition under those conditions. It is the reprecipitated material that is detected as indicating the end-point. The sensitivity found better than 500 μg in 0.6 L.

Wax-lined glass vessels

Given the difficulties which emerged, solubilities for CaF2 and FAp were redetermined using vessels coated with wax (paraffin wax, "congealing point" about 55℃, BDH Poole, England), as well as checks for HAp itself under the same conditions. A thin coating of wax was prepared by rinsing with a solution of approximately 5 g wax/0.5 L acetone, and allowing the solvent to evaporate. All glass surfaces except the

pH electrode membrane and salt bridge pin were treated. This was achieved by holding the electrode horizontally, or inverting it as appropriate, and by painting the solution onto the stem but avoiding the critical areas.

Numerical analysis

RAMESES[15], a computer program (in Basic PDS, v 7.1, Microsoft, Redmond WA, USA) for resolving multiple equilibrium equations, was used to calculate S [CaF2].

RESULTS

CaF_2

The experimental solubility isotherm of CaF_2 in 100 mM KCl determined in an uncoated vessel is shown in Figure 1. This was problematic in several respects, deviating substantially from the calculated line over the whole range covered. This and other data for FAp and HAp with [F], as well as observation of the vessel walls after a sequence of determinations, led to the postulation of interference and the use of wax-coated apparatus. For CaF_2 (Figure 2), the results then (filled circles) were as theoretically-expected at low pH values, although the decrease at pH > 8 was inconsistent (in position and slope) with the formation of Ca $(OH)_2$.

FAp

Anomalous results were also obtained for FAp in uncoated vessels, with apparently higher solubility than HAp[6] at pH > 4.4 (Figure 3). Results obtained using wax-coated apparatus (Figure 4; filled circles) showed that FAp was just slightly less soluble than HAp over the whole range examined. An abrupt change of slope of the solubility isotherm (similar to that for HAp) occurred at pH approximately 3.9.

HAp in the presence of fluoride

An anomalous result for HAp in the presence of 1 mM fluoride was also obtained in uncoated glass apparatus, with a substantially higher apparent solubility than pure HAp[17] at pH > 4.6 (data not shown). The results obtained in coated apparatus are shown in Figure 4 (dotted circles). The apparent solubility was then, as expected, slightly less than for HAp in 100 mM KCl alone. At pH 4, the solubility was approximately one half in the presence of the fluoride, the difference vanishing by the stoichiometric equivalence point at 1 mM HAp, again as would be expected.

HAp

The solubility of HAp at low pH (Figure 4; quartered circles) and in a wax-lined vessel (Figure 4, crossed circles) was consistent with Zhu *et al*[18]. suggesting that the S[HAp] determined then was unaffected by the exposure to the glass, and that it extends uniformly to at least pH 2.75, overlapping the range covered for FAp.

DISCUSSION

It is widely known that aqueous solutions containing hydrofluoric acid (i.e., fluoride ions) at low pH etch or dissolve glass surfaces by the formation of tetrafluorosilane (SiF_4)[19]. The reaction may be expressed as:

$$SiO_2 + 4HF(aq) \rightarrow SiF_4 + 2H_2O$$

It is not known what the pH-dependence of this reaction is, but clearly there is a risk that needs consideration. In this connection, Saxegaard *et al*[20]. and McCann[21] reported (without explanation) that their works were carried out in plastics test tubes, others[22,23] have made no mention, possibly due to an assumption of no effect given the moderate pH values used. Thus, the significant errors encountered in this titration, especially at high pH, is a critical finding, in particular because borosilicate glass is commonly considered essentially inert; other glasses might be expected to be more reactive. Not only was corrosion of the glass surface suspected, but also precipitation nucleated on the walls of the glass vessel may have distorted the results.

Interference by the glass of the vessel may therefore have affected other published results where the solution contained fluoride. The validity of any pK_{sp} obtained in such a case must be reconsidered, in addition to concerns about phase transformations.

Coating the apparatus with paraffin wax appears to be a good resolution of the problem, the working temperature of 37℃. The solution was flushed with nitrogen, monitored by pH electrode being well below the 'congealing' point of the wax (55℃. The solution was flushed with nitrogen, monitored by pH electrode). On setting up the apparatus with a test solution, air bubbles initially easily-attached to the surface (from splashing during filling and coming out of solution on warming), although these disappeared after several hours (during the normal stabilisation period), and a stable baseline laser signal output was obtained. There is no expectation of any chemical interaction between the test system and the paraffin wax, and the experimental check of HAp solubility determined in such wax coated apparatus which gave values that matches previous results of Zhu *et al*[18]. very well (Figure 4; crossed circles). It is concluded that work in such a wax-coated apparatus is consistent and reliable. It is to be noted that while plastics vessels could be used, for the present technique optical windows are required, and the glass of the pH electrode body still requires treatment.

The solubility isotherm determined for CaF_2 was consistent with the calculation of RAMESES (Figure 2). This may be compared with the value of 10.45 reported by McCann[21]. Also shown in Figure 2 are experimental results in comparison with McCann[21] and Larsen[23] outcomes in water and KCl solutions, although only covering a narrow range from pH 4 to 8, corresponding to the plateau here; the discrepancy is not large. However, it may be noted that the nature of the vessel used by Larsen was not mentioned, and solid was in contact with the solution for just 3 h. Below pH 4, the solubility rises, in keeping with the predicted line, essentially due to the formation of the species HF in solution. But, above pH 8, the apparent solubility falls rapidly, although this does not correspond to the formation of Ca $(OH)_2$, which would only be expected from pH approximately 11. The descending limb is consistent with the formation of the now postulated new solid species CaFOH (apparently not attested in the literature), taking a value for the formation constant of approximately 0.1012. Formal confirmation of the existence of this solid is required, although it is apparent that the titration technique is capable of detecting otherwise obscure behaviour with considerable sensitivity. Even so, CaFOH may not be involved in saliva or tooth tissue chemistry, unless the pH rises above approximately to 8 (which, for example, would commonly happen during the use of an alginate impression material, although fluoride-containing toothpastes may achieve this - a point worth further study). Nevertheless, the concordance of the calculated and experimental solubility isotherms of CaF_2 below that point is taken as further confirmation that titration is sound.

The S[FAp] is nearly parallel to that previously reported for HAp[18], being just slightly less soluble (Figure 4) over the whole pH range examined. In particular, the line lies about 0.2 log unit below that of HAp (i.e., X approximately 0.63). Previously, the pK_{sp} values for FAp and HAp have been reported as 60.5[21] and 58.4[12], respectively. Given that the S[HAp] shown in Figure 4 corresponds at low pH to a value of approximately 63[18], for FAp now the value would be approximately 64 at pH approximately to 3.9, but only if the effective concentrations of fluoride and hydroxide ions were equal; preliminary calculations suggest that this is unlikely. A detailed calculation has not been attempted because of the speciation difficulties mentioned below. The slight convergence apparent between S[HAp] and S[FAp] may be attributable to the formation of solution species such as HF, as above for CaF_2, having an increasingly important effect.

A convergence at pH approximately to 3.6 in the case of Moreno's data is registered; even so, there is a noteworthy parallel between his data and that of the 'unwaxed' results in Figure 3. The difference is probably, at least in part, due to unrecognised solid-state phase transformations arising from non-congruent dissolution, but it may also be related to the use of phosphoric acid solutions for pH adjustment. Despite claims that Ca-P solution species had been taken into account in the calculations; it is evident from the gross discrepancy that there is a problem with one or more assumptions. The simple phenomenology of this titration is believed to be more reliable and underlines the need for very detailed solution speciation studies. It is notable in particular that there was no evidence of the formation of CaF_2 in the FAp titration here, contrary to McCann's assertion[21] that it forms when FAp is dissolved in a solution below pH 4.5.

The very close coincidence of the pH (i.e., 3.9) at which the change of slope occurs in both S [HAp] and S [FAp] is taken as strong evidence

that the crystal chemistries of the two precipitates are similar. In addition, the decreasing Ca/P ratio with decreasing pH for FAp for pH 3.2-4.1 is consistent with the similar observation made for HAp[18] (indeed, the values are indistinguishable), again presumably due to the incorporation of HPO_4^{2-}, stressing that no phase transformation was detected across pH 3.9. Even so, particle morphology appears to change at the lower pH values of 3.6 and 3.2 in the present case.

As expected, the solubility of 1 mM [HAp] in the presence of 1 mM [F] is indistinguishable from that of 1 mM [FAp], which point lies at pH approximately 3.2 (Figure 4). On the other hand, the suppression of S [HAp] at high pH is relatively small in comparison with the discrepancy between the titration S [HAp] and previously reported solubility data, as discussed earlier[18]. The fear that minute contamination with fluoride (although then shown to be unlikely) might be responsible for a discordant result is thereby allayed, given that the addition here (1 mM) is three hundred-fold that of the HAp at saturation at pH 5. It is not yet known whether FAp nucleation provides a template for (epitaxial) growth of HAp, or whether a true solid-solution gradient of [F-] exists within each crystal, as might be expected[24]. Experimental difficulties are anticipated in the preparative process necessary to handle this.

CONCLUSION

The interference from contact of fluoride-containing test solutions with glass is a critical concern that implies the need for rechecking previous F-related solubility data. However, wax-coated apparatus provides a simple means of avoiding trouble.

The matching of experimental and numerical S [CaF_2] s reconfirms the validity of titration. The identification of a new solid species, CaFOH, requires confirmation but suggests that other solubility subtleties may be found by such means.

S [FAp] is confirmed to lie just below and nearly parallel to S [HAp], with both similar Ca/P - pH dependency and slope discontinuity at pH 3.9. CaF_2 was not detected in the equilibrium. The role of solution fluoride in modifying the apparent solubility of HAp has been demonstrated.

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