SHORT COMMUNICATION

A Descriptive Study - In Vitro: New Validated Method for Checking HAp and FAp Behaviours

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ABSTRACT

Background: **Owing to the topical fluoride treatment, calcium fluoride and fluorapatite (FAp) are forming on a dental enamel surface. Many in oral biology, prevention and treatment have studied hydroxyapatite (HAp) and FAp dissolution. Some focused on precipitation reactions since these materials are prerequisites in dental hard tissues. Nonetheless, FAp is less soluble phase amongst calcium phosphates and has more complicated equilibria.**

Objective: **This study aims to validate a suitable method for checking HAp and FAp behaviours.**

Design: **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. A comparative study of the solubility of the CaF_2, HAp, and FAp were performed among wax-coated apparatus and an uncoated container experiment.**

Results: **These 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.**

Conclusions: **The titration was successfully validated where wax-coated method had avoided any interference with F^--containing test solutions.**

KEY WORDS

biocorrosion, complicated equilibria, particles, phase, topical fluoride

INTRODUCTION

The solubility of fluorapatite (FAp, $Ca_5(PO4)_3F$) and CaF_2 are important in understanding their formation, prevention and treatment[1)]. 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[1)]. Noting that titration does not depend on equilibration with solid addition materials, but the complete dissolution of each increment[2,3)]. For FAp, more complicated equilibria may be involved due to the presence of fluoride. Farr and Elmore[4)] calculated the inversely related to the solubility constant (pKsp = -logKsp) 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, would be of value. However, the previously noted difficulties arising from lack of detailed solution equilibria for the relative calculation for HAp leads to a discrepancy between experiment and theory. 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, 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].

Table 1. With FAp titrant, elemental composition of the precipitate analysed by EDX.

pH	Ca/P	Ca	P	F	O
3.2	1.47 ± 0.05	19.51	13.30	5.23	61.96
3.6	1.51 ± 0.05	20.24	13.39	5.50	60.87
4.1	1.62 ± 0.05	21.24	13.08	5.60	60.08
FAp	1.65 ± 0.05	23.11	13.97	5.64	57.28

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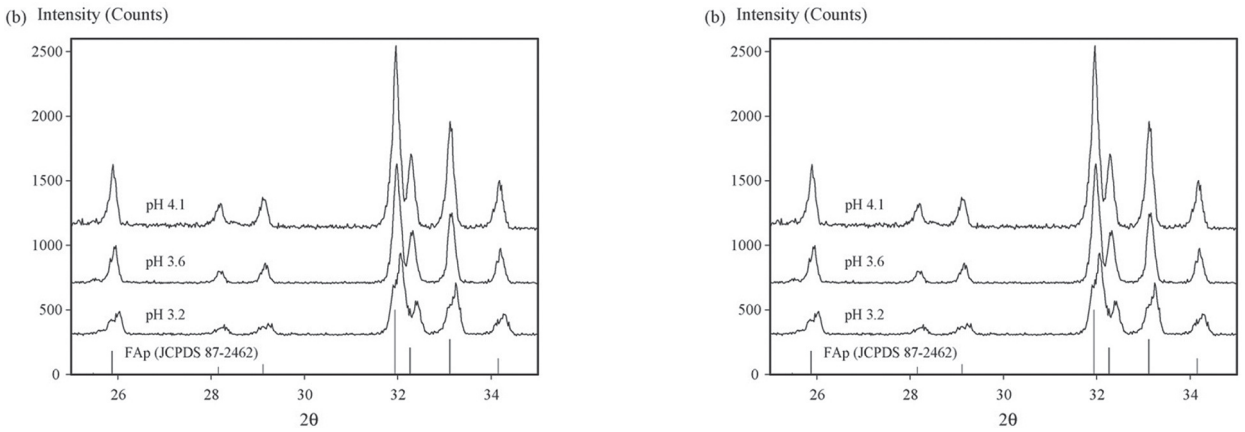

Figure 1. XRD patterns for "preparative" precipitated solids in FAp titration compared with standard XRD of FAp and CaF_2; (a) scanning angle from 10° to 70°, (b) scanning angle from 25° to 35°.

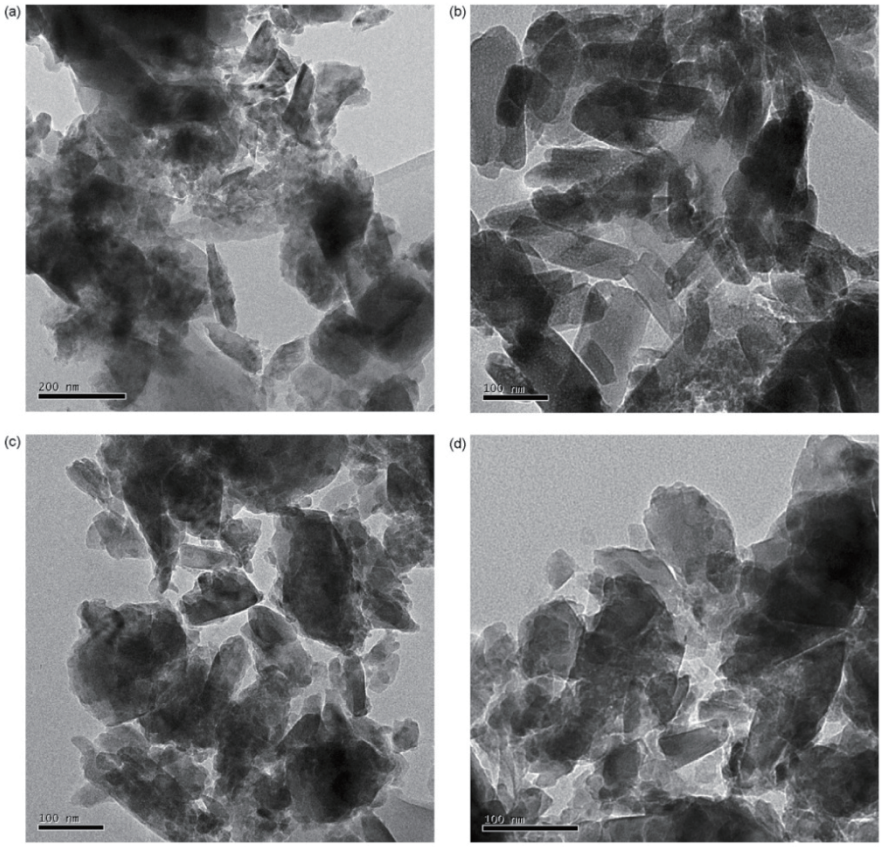

Figure 2. TEM images of (a) original titrant FAp and precipitated particles from the solution at (b) pH 4.1, (c) pH 3.6 and (d) pH 3.2.

MATERIALS AND METHODS

Given the difficulties which emerged, the solubility for CaF_2 and FAp were redlined using vessels coated with wax (paraffin wax "C_nH_{2n+2}, n: 20-30", congealing point: about 55℃, BDH Poole, England). Checks were performed for HAp itself under the same conditions. A thin coating of wax was prepared by rinsing with a solution of approximately 1 g wax/0.1 L acetone ($(CH_3)_2CO$), 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.

Solid formation and characterisation

As for HAp, to acquire sufficient material for analysis by X-ray diffraction (XRD), energy-dispersive X-ray analysis (EDX) and transmission electron-microscopy (TEM), a slight excess of solid FAp was added in small portions to the solution after reaching the titration endpoint at three pH values (3.2, 3.6, and 4.1), allowing time (around 10 days) after each for dissolution, reprecipitation and equilibration, including grain growth. The composition of the prepared precipitate was characterised by XRD (Model D/max 2550 V, Rigaku, Tokyo, Japan) using Cu K_a (l = 0.15406 nm) radiation in step-scan mode (2_θ = 0.02° per step). The Ca/P ratio was determined by elemental analysis using EDX (LEO 1530 FESEM, Oxford Instruments, Oxford, UK). Oxygen and fluorine were also determined. The precipitate morphology was observed by field-emission scanning TEM (Tecnai G2 20 STEM, FEI, Hillsboro OR, USA).

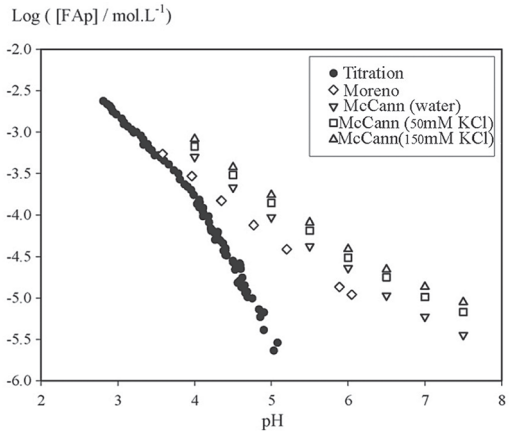

Figure 3. Comparison of some literature data for FAp solubility with the present titration results (McCann[8] and Moreno *et al*[9]).

RESULTS

XRD - TEM analysis

XRD of the precipitate indicated that no phase other than FAp was presented at pH 3.2, 3.6 or 4.1 (Figure 1a). As with HAp[5], the crystallinity decreased slightly with the decrease of pH, and XRD peak positions also deviated slightly from standard FAp at pH 3.2 and 3.6 (Figure 1b). EDX showed that Ca/P ratio of the precipitate decreased steadily with pH (Table 1). At pH 4.1, particles appeared to be single crystals with rod-like morphology (Figure 2b), while at pH 3.6 (Figure 2c) and 3.2 (Figure 2d) they appeared to be more polycrystalline.

Solubility of HAp in excess F^-

An anomalous result for HAp in the presence of 1 mM F- was also obtained in uncoated glass apparatus, with a substantially higher apparent solubility than pure HAp at pH > 4.6 (data not shown). The apparent solubility was, as expected, slightly less than for HAp in 0.1 M KCl alone. At pH 4, the solubility was approximately 1/2 in the presence of the F^-, the difference vanishing by the stoichiometric equivalence point at 1 mM HAp, again as would be expected.

DISCUSSION

It is widely known that aqueous solutions containing hydrofluoric acid (HF) (i.e., F^-) at low pH etch or dissolve glass surfaces by the formation of tetrafluorosilane (SiF_4)[6]. The reaction may be expressed as in Equation 1:

$$SiO_2 + 4HF_{(aq)} \rightarrow SiF_4 + 2H_2O \quad (1)$$

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[7] and McCann[8] reported (without explanation) that their works were carried out in plastics test tubes, others[9-11] have made no mention, possibly due to an assumption of no effect given the moderate pH values used. Thus, the significant errors encountered now 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 container may have distorted the results.

Interference by the glass container may therefore have affected other published results where the solution contained F^-. 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℃ being well below the 'congealing' point of the wax (55℃). 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*.[12] very well. It is concluded that work in such a wax-coated apparatus is consistent and reliable. It is to be noted also that while plastic 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 determined for CaF_2 was consistent with the calculation of RAMESES, using a visually-fitted value for $pK_{sp}[CaF_2]$ = 10.3 (i.e., log(β), Table 1). This may be compared with the value of 10.45 reported by McCann[8].

It may be noted that the nature of the container used by Larsen was not mentioned, and the solid was in contact with the solution for just 3 h. Below pH 4, the solubility rises, essentially due to the formation of HF species in the 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 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 F^--containing toothpastes may achieve this - a point worth further study). Nevertheless, the concordance of the calculated and experimental solubility of CaF_2 below that point is taken as further confirmation that titration is sound.

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 lower solubility for FAp was obtained over the range by titration compared with the usual large-excess of solid added materials method[9] (Figure 3), although there is convergence at pH approximately to 3.6 in the case of Moreno's data. 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 (H_3PO_4) 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, contrary to McCann's assertion 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 at pH 3.2-4.1 is consistent with the similar observation made for HAp[12] (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.

The presence of 1mM KF appreciably affected the apparent solubility of HAp, presumably due to the formation of FAp (because of the extremely small quantities involved, XRD confirmation has not yet been possible). 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[12]. The fear that minute contamination with F^- (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[13].

The health-care and economic consequences of F^- in the oral context are profound, yet the whole question of calcium phosphate solubility -

the benchmark, if you will - remains unsatisfactorily resolved. The chain of reasoning is long and convoluted. Detailed understanding of the solution chemistry is essential for being able to control laboratory work, and thus the efficacy of topical treatment, to say nothing of the interpretation of epidemiological data related to the fluoride content of tooth tissue.

CONCLUSION

The interference from contact of F^--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 F^--containing solution in modifying the apparent solubility of HAp has been demonstrated.

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