SOLUBILITY BEHAVIOR OF CARBONATED APATITE
IN THE PRESENCE OF SOLUTION FLUORIDE

by

Hong Zhuang

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Doctor of Philosophy

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SUPERVISORY COMMITTEE APPROVAL

of a dissertation submitted by

Hong Zhuang

This dissertation has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

Chair:

Bradley D. Anderson

G. Ann Powell
To the Graduate Council of the University of Utah:

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Approved for the Major Department

Jindrich Kopecek
Chair/Dean

Approved for the Graduate Council

David S. Chapm
Dean of The Graduate School
ABSTRACT

The objective of this study was to quantitatively understand the solubility properties of carbonated apatite in the presence of solution fluoride.

The first project of this research was to assess the applicability of the Metastable Equilibrium Solubility (MES) concept for the study of CAP solubility behavior over a range of pH and a wide range of solution fluoride concentrations, and to examine the hypothesis that in the presence of solution fluoride, a surface complex with the stoichiometry of fluorapatite (FAP) governs the MES. The findings of this study demonstrated that the CAPs investigated exhibit the MES distribution phenomenon in solution media of varying pH and fluoride concentrations. The experimental MES data obtained with these CAP preparations at the lower pH (4.5) and at higher solution fluoride levels (≥ 0.1 ppm) suggested that in the presence of solution fluoride the MES governing surface complex may be an entity possessing a stoichiometry of FAP.

Previous studies have shown that the MES behavior of CAPs may be described by a surface complex with the stoichiometry of hydroxyapatite (HAP) and that the magnitude of the MES is determined by the CAP crystallinity. In the presence of solution fluoride, employing the results that a surface complex of FAP stoichiometry controls the dissolution of CAPs, the MES distributions of CAP preparations of various solubilities can be determined. In this study, it has been found that there is a linear
relationship between the CAP solubility and the CAP crystallinity parameter and that the slope of the CAP mean $pK_{\text{HAP}}$ and the mean $pK_{\text{FAP}}$ are essentially parallel.

Knowledge of the lowest concentrations (thresholds) of solution fluoride which influence the MES of CAPs is critical in understanding the mechanism and stoichiometry controlling the solubility of CAPs at extremely low solution fluoride levels. The major finding of this third study is that the influence of fluoride on the CAP solubility becomes asymptotically negligible as solution fluoride is reduced to ultra-low levels, i.e., $\leq 0.02$ ppb. Also, at these low fluoride levels (i.e., $\leq 0.02$ ppb), the solubility governing function is consistent with a surface complex possessing the HAP stoichiometry.
To my wife
Yin,
and my parents
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CHAPTER 1

INTRODUCTION

Major proportions of human dental enamel (~95% wt) and bone (~65% wt) are inorganic or mineral content (1, 2), and structural refinement based on biological mineral and synthetic apatite (3) has revealed that the mineral phase in teeth and bones resembles hydroxyapatite (HAP), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. For biological hard tissues such as dental enamel and bone, the major inorganic components (see Table 1.1) are calcium and phosphorus; the minor constituent is carbonate (a few percent by weight); and trace elements are sodium, magnesium, potassium, fluoride, strontium, chloride, etc. Since demineralization and remineralization of biologically hard tissues are important for the physiological function and pathological processes of human beings, e.g., dental caries and osteoporosis, studies have been done for over a century in order to understand the physicochemical properties of biological apatites. Possibly because of the complexity of the composition of these apatites, up to now, satisfactory results have not been obtained yet. The general goal of this laboratory’s research is to quantitatively understand the solubility behavior of biological and synthesized apatites so that essential understanding can be developed for the processes of biomineral disorder diseases so that effective therapeutic methods can be found for the prevention and treatment of these diseases.
Table 1.1* Composition of inorganic phases of adult human enamel and bone.

<table>
<thead>
<tr>
<th>Composition (% wt)</th>
<th>Enamel</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, Ca(^{2+})</td>
<td>36.5</td>
<td>34.8</td>
</tr>
<tr>
<td>Phosphorus, as P</td>
<td>17.7</td>
<td>15.2</td>
</tr>
<tr>
<td>(Ca/P) molar</td>
<td>1.63</td>
<td>1.71</td>
</tr>
<tr>
<td>Carbonate, CO(_3^{2-})</td>
<td>3.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Sodium, Na(^+)</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Magnesium, Mg(^{2+})</td>
<td>0.44</td>
<td>0.77</td>
</tr>
<tr>
<td>Potassium, K(^+)</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>Fluoride, F(^-)</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Chloride, Cl(^-)</td>
<td>0.30</td>
<td>0.13</td>
</tr>
<tr>
<td>Pyrophosphate, P(_2)O(_7^{4-})</td>
<td>0.022</td>
<td>0.07</td>
</tr>
<tr>
<td>Total</td>
<td>97.0</td>
<td>65.0</td>
</tr>
<tr>
<td>Organic matrix</td>
<td>1.5 (Keratin and other proteins)</td>
<td>25.0 (Collagen and other proteins and lipids)</td>
</tr>
<tr>
<td>Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoclasts, osteoblasts, lining cells, and osteocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>1.5</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* This table has been revised from (4).
Biological apatites are microcrystalline, nonhomogeneous, and nonstoichiometric in nature, e.g., Ca-P molar ratio for enamel $< 1.67 < \text{Ca-P molar ratio for bone}$ (see Table 1.1). Also, minor and trace inorganic elements are present in biological apatites which may be either incorporated into the apatite lattice, adsorbed onto the apatite crystal surface, or both. Because of the complexity and variation in the composition of actual biological mineral, in vitro studies on synthetic apatites are convenient to increase our knowledge of the physicochemical properties of tooth enamel and bone mineral. The solubility behavior of hydroxyapatite, the least complicated biological apatite which possesses a Ca-P molar ratio of 1.67, has been intensively studied for several decades. To date, controversies still remain as to whether hydroxyapatite is an appropriate model for studies of biological hard tissues (4, 5). Carbonate is the major foreign anion in mature human dental enamel (about 0 to 3.5 weight %) and bone mineral (about 6 to 7.5 weight %), and it has been found to have a strong preference to incorporate into hydroxyapatite, thus forming carbonated apatite (CAP). Budz et al. (5) suggested that the dissolution of dental enamel more closely parallels that of carbonated apatite than that of hydroxyapatite. Studies in our laboratory (6, 7) have shown that carbonated apatite, not hydroxyapatite, is a more appropriate synthetic model for human dental enamel because of its similar response to thermal treatment. In addition, Baig et al. (8) have found that carbonated apatite and bone mineral are more similar in chemical composition, X-ray diffraction pattern, FTIR spectra, and solubility behavior. These findings provide evidence that CAP serves as a better model system for enamel and bone studies. For this reason, carbonated apatites synthesized in this laboratory have been employed for conducting research on solubility properties.
Fluoride has been used as a therapeutic reagent for the prevention and treatment of dental caries for several decades. It has been widely accepted that dental decay is basically a process of dissolution of enamel mineral attacked by organic acid generated by metabolic processes of oral bacteria. Epidemiological studies conducted over half a century, starting in the early 1940s (9), showed an inverse relationship between the concentration of fluoride in drinking water and caries incidence. In the meantime, fluoride therapy has also been promoted to be an effective means to increase bone mass in the cure of crush fractures due to postmenopausal osteoporosis. Studies have also disclosed that fluoridation of public drinking water may protect against the development of osteoporosis. For instance, Leone et al. (10) found a reduced occurrence of vertebral osteoporosis in Bartlett County, Texas, with a fluoride content in drinking water of 8 parts per million (ppm) compared with that in Farmington, Massachusetts, with a fluoride content in drinking water of 0.09 ppm. Pak et al. (11) concluded that slow release of fluoride could inhibit new vertebral fractures and augment spinal and femoral neck bone mass.

To date, it is generally believed that the dissolution of tooth and bone mineral by organic acid is the major cause of caries and osteoporosis. As a result, understanding of the solubility properties of mineralized tissues in the presence of fluoride is considered to be prerequisite to a quantitative understanding of the processes of inhibitory effect of fluoride on dental caries and osteoporosis. Also, this understanding can be utilized for the design and development of effective fluoride delivery systems for the prevention and treatment of dental decay and osteoporosis.
The mechanism of fluoride effect on the solubility behavior of enamel and bone mineral has been extensively investigated over the past decades. Since fluoride concentration in drinking water has been found to be inversely related to the occurrence of dental caries and osteoporosis and to be directly correlated with the fluoride content of tooth enamel (12) and bone mineral (13), it was once presumed that the reduction of incidence of dental caries and osteoporosis came from the incorporation of fluoride ions into the apatite lattice and the formation of less soluble crystals. Although some results suggested that fluoride-containing apatites have lower solubilities compared to apatites without fluoride (14, 15), most results (16-19) have shown that the reduction of solubility resulting from incorporation of fluoride is not sufficient to account for the dramatic reduction role of fluoride on caries and osteoporosis. These results demonstrated that it was solution fluoride that could significantly reduce dissolution rate of apatites, even when the fluoride levels in solutions were low. Based on numerous studies (16, 17, 19), it has been generally recognized that fluoride in solution plays a dominant role in demineralization and remineralization of dental enamel and bone mineral. Although several reasons have been used to account for the inhibitory effect of solution fluoride on the solubility of apatite, up to now there is not any proposed mechanism that is widely accepted. Based on this general uncertainty, the present research focuses on the solubility behavior of carbonated apatite in the presence of solution fluoride, with an aim to achieve mechanistic understanding of solution fluoride influencing the solubility of biological and synthetic apatites.

All the carbonated apatite samples, tooth, and bone minerals thus far studied in our laboratory, starting from Hsu et al. in 1994 (20), have exhibited metastable
equilibrium solubility (MES) phenomena. As a subsequent study in this laboratory, the first project of this dissertation was to investigate whether the MES phenomenon holds for CAPs in the presence of solution fluoride (see Chapter 4). Furthermore, the functional form (ion activity product stoichiometry) governing the solubility (MES) of CAPs in the presence of solution fluoride was systematically examined in this project. Then the results obtained have been employed to determine the magnitudes of MES distributions for different CAP preparations, and the mean values of the MES distributions are utilized as the measurement of apparent solubilities for the CAPs. A linear relationship has been found between the CAP mean MES and crystallite disorder (crystallinity) and such experiments and results are presented in Chapter 5. The last study of this dissertation has concerns the lowest concentrations of solution fluoride which affect the MES behavior of the CAPs. Knowledge of the threshold levels of solution fluoride is important in CAP solubility studies when CAP MES behavior is to be examined without the influence of fluoride, and this work is shown in Chapter 6.
References


CHAPTER 2

REVIEW OF THE LITERATURE

During the past several decades, solubility behavior of biological and synthetic apatites has been intensively studied. In the meantime, much effort has been devoted to revealing the correlation between fluoride and the physicochemical properties of apatite minerals. The importance of fluoride mainly comes from the fact that fluoride is an effective agent for the prevention of dental caries and a potential means for the treatment of osteoporosis. This literature review pertaining to the relationship between solubility behavior of carbonated apatite and fluoride is divided into the following four parts:

**Solubility Behavior of Apatites**: the solubility behavior of hydroxyapatite will be discussed and the focus will be on the thermodynamic solubility product of hydroxyapatite.

**Effect of Carbonate on Apatites**: a general view of the properties of carbonate-containing apatite which make it an appropriate model for biological apatites will be presented.

**Effect of Fluoride on Apatites**: Some proposed mechanisms for the fluoride inhibiting effect on the dissolution of apatites will be addressed.

**Fluorine Analysis Methods**: recently developed fluorine analysis techniques will be reviewed, and the advantages and limitations of the methods will be discussed.
Solubility Behavior of Apatites

Apatite refers to a group of minerals of general chemical formula of Ca$_5$(PO$_4$)$_3$(CO$_3$,OH,F), and the name comes from the Greek *apate* (deceit) because the mineral was often mistaken for more valuable gems like beryl and olivine. Due to the complexity and variation associated with biological apatites (1-4), e.g., tooth enamel and bone mineral, it has been necessary to study synthetic analogs in order to gain insights into the composition and structure of the more complex biological minerals, as well as to increase knowledge on their solubility and adsorption properties. Calcium phosphates are deposited (calcified) in the body in normal and pathological conditions (5, 6) forming minerals such as hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, HAP), dicalcium phosphate dihydrate (CaHPO$_4$·2H$_2$O, DCPD), dicalcium phosphate (CaHPO$_4$, DCP), octacalcium phosphate (Ca$_8$H$_2$(PO$_4$)$_6$, OCP), and tricalcium phosphate (Ca$_3$(PO$_4$)$_2$, TCP). X-ray and neutron diffraction analyses (7, 8) demonstrated that the structure of HAP is closely similar to that of the inorganic phases of teeth and bone. It was also found that among the calcium phosphates described above, HAP is the most stable phase and has the lowest solubility at physiological pH. Consequently, HAP was initially utilized as the prototype material for the principle crystalline mineral in tooth and bone.

Numerous studies have been conducted to investigate the solubility of HAP in the past century. Depending on the method of HAP sample preparation, the thermodynamic ion activity products (pK$_{sp}$) obtained varied from about 98 to 118 (9-11). Other published values of the negative logarithm of the solubility product of HAP reported in the literature are: 115.5 by Clark (12), 117.1 by Verbeeck et al. (13), and 119.9 by Wier et al. (14). Therefore, from different studies (using different pH, HAP synthesis method,
slurry density (solid-to-solution ratio), etc.), researchers have had difficulty obtaining a consistent solubility product for HAP. This variation is about 10 times larger than the expectation if surface energy effects due to particle size were taken into consideration. The fact that the solubility product of HAP has been controversial raises an important question: whether the thermodynamic equilibrium solubility principle can be applied to the solubility study of HAP.

Based on studies of enamel powder, Patel and Brown (15) warned that the assumption of a constant solubility product for enamel was an oversimplification that should be avoided. They further concluded that the most probable cause of the variable solubility product of enamel mineral was the presence of varying amounts of structural defects and impurities in the enamel crystals. The fact that apatites possess varying degrees of crystallite disorder may be used to interpret the inconsistency of the reported solubility product values of synthetic HAP. It was found that perfect HAP crystals are very difficult to synthesize in laboratories. Brown (15) reported that HAP synthesis processes were slow or ineffectual under many conditions. Like dental enamel, impurity ions are easily incorporated into the HAP crystal structure during synthesis. Young and Holcomb (16) reported that HAP preparations from different methods had large differences in particle size, HPO$_4^{2-}$ and CO$_3^{2-}$ content, lattice parameters, vacancies, etc. Therefore, effort to obtain a consistent thermodynamic solubility product of HAP may not be an appropriate route to study the solubility properties of synthetic apatites.

Starting from Hsu et al. (17), our laboratory has found that the metastable equilibrium solubility (MES) phenomenon holds for synthetic apatites, tooth enamel, and bone mineral. A description of the MES phenomenon is as follows: (1) apatites reach a
metastable equilibrium state, not true thermodynamic equilibrium, with aqueous solution from several hours to several days and maintain this metastable equilibrium state for a very long time (months or longer); (2) if the solution is undersaturated with respect to an apatite crystal, the crystal will dissolve; if the solution is supersaturated with respect to the apatite crystal, dissolution will practically stop while no precipitation is observed; and (3) apatite is composed of crystal domains which possess varying degrees of crystallite disorder or defects. As a result, some apatite crystal domains have a high solubility (MES) whereas others have a low solubility, and the apatite taken as a whole demonstrates a distribution of MES. All synthetic apatites studied thus far in our research group (18-20) exhibit MES phenomena in acetate buffers at pH 3.5 - 6.5. With this MES concept, the inconsistent values of the reported solubility product of HAP at different test pH and slurry density conditions can be reasonably interpreted.

Effect of Carbonate on Apatites

Carbonate, the chief impurity ion in biological apatites, is found to incorporate naturally into the HAP structure, forming carbonated apatite (CAP). LeGeros et al. (21) has shown that carbonate can substitute for either phosphate or hydroxide in the HAP crystal lattice. When CAPs are prepared at high temperatures (~1000 °C) under dry conditions, carbonate substitutes for hydroxide to form type-A CAP. When CAPs are prepared from aqueous systems by precipitation or hydrolysis methods at 37–100 °C, carbonate substitutes for phosphate and sodium substitutes for calcium, forming type-B CAP (21). X-ray diffraction and IR spectroscopy have shown dental enamel and bone mineral to be type-B CAP. Therefore, research in our laboratory has concentrated on type-B CAP and, for simplification, CAP refers to type-B CAP in this dissertation.
CAP may be described by the formula: \( \text{Na}_x \text{Ca}_{10-x}\{(\text{PO}_4)_{6-x}(\text{CO}_3)_x\}(\text{OH})_2 \), where \( x \) represents the extent of carbonate incorporation into HAP. The substitution of carbonate for phosphate causes the hexagonal crystal structure of HAP to be disturbed. The following main differences have been found for CAP compared to carbonate-free HAP (22, 23): (a) decrease in \( a \)-axis and increase in \( c \)-axis dimensions, (b) decrease in crystal size, (c) increase in crystal strain, (d) increase in solubility. Through X-ray diffraction analysis by Rietveld method, Baig et al. (24) have recently shown that carbonate in apatite increases the apparent solubility (MES) by increasing the crystallite disorders or defects, which can be characterized by the crystallinity parameter microstrain.

Since biological apatites contain a substantial amount of carbonate (see Table 1.1 in Chapter 1), it seems logical to study CAP as a model compound for enamel and bone mineral. Budz et al. (25) addressed how the dissolution behavior of human enamel is more similar to that of CAP than HAP. Studies in our laboratory (26, 27) reported that the responses of CAP samples to laser irradiation and heat treatment are similar to those of tooth enamel. X-ray diffraction and FTIR spectra experiments (20) have also illustrated the similarity of CAPs and bone minerals. Moreover, the solubility of biological apatites has been found to be more similar to that of CAP than the solubility of HAP. All these studies suggest that CAP is a better synthetic model compound than HAP and, therefore, synthesized carbonated apatites have been used as the prototype to investigate solubility behavior of enamel and bone minerals in this study.

**The Effect of Fluoride on Apatites**

Epidemiological studies (28-30) showed an inverse relationship between the concentration of fluoride in public drinking water and incidence of dental caries and
osteoporosis. An analysis made by Backer Dirks (31) indicated that a caries reduction of approximately 50% was observed when fluoride in the drinking water was 1 ppm. Investigations by Bernstein et al. (32) found that prevalence of vertebral fractures among women was lower in regions of North Dakota where drinking water was fluoridated than in regions which were not fluoridated. Since it has been generally found that the fluoride content of enamel and bone reflects the levels of fluoride in drinking water, it was assumed that the incorporation of fluoride into the crystalline lattice of tooth and bone mineral could increase the crystallite stability, which would be demonstrated by forming low-soluble crystallites of biological apatites. However, many studies (33, 34) did not find a correlation between caries and fluoride content in enamel. Instead, the results have suggested that the extent of fluoride incorporation into enamel crystallite may not be the primary factor but fluoride in solution phase, even at low concentrations, may play an important role of the cariostatic effect of fluoride. These studies indicate that more than one role of fluoride may be responsible for the fluoride inhibitory effect on the dissolution of apatitic minerals.

The incorporation of fluoride into carbonated apatite crystals brings the substitution of F\textsuperscript{-} for OH\textsuperscript{-}, which can be best described by the following chemical formula (35): Na\textsubscript{x}Ca\textsubscript{10-x}(PO\textsubscript{4})\textsubscript{6-x}(CO\textsubscript{3})\textsubscript{x}(OH)\textsubscript{2-y}F\textsubscript{y}. Fluoride-containing apatites have the following main changes in property compared to F-free apatites (35): (a) contraction in the a-axis but no change in the c-axis dimensions, (b) increase in crystal size and decrease in crystal strain, (c) calcium-phosphate ratio approaching the stoichiometric value of 1.67, (d) decrease in solubility. In order to address the question of how the incorporation of fluoride into the crystalline lattice influences the solubility of apatites, Moreno et al. (36)
investigated the solubility of fluoridated hydroxyapatites which possessed a general formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-y}\text{F}_y$, where $0 \leq y \leq 2$ with $y = 0$ represents hydroxyapatite and $y = 2$ represents fluorapatite. They found that there was a steady decrease in the solubility of fluoridated hydroxyapatites with increasing values of the degree of substitution up to about 60%, i.e., the corresponding $y$ value being about 1.2. Aasenden et al. (37) have found that the fluoride content of enamel was 3000 ppm and 2100 ppm in communities where the drinking water was fluoridated (1 ppm) and non-fluoridated (< 0.1 ppm), respectively. Based on the results of Moreno et al. (36) as discussed above, a concentration of 3000 ppm and 2100 ppm fluoride in enamel corresponds to a degree of fluoride substitution of 8% and 5.6%, respectively, but this difference in the content of fluoride incorporation into the enamel crystalline lattice would not result in a significant difference in enamel solubility, which was assumed to account for the observed difference of caries incidence. So the incorporation of fluoride into enamel apatite lattice may not be effective enough to prevent teeth from decaying. A similar result was also obtained by Ogaard et al. (38) by exposing shark enamel (consisting of carbonated fluorapatite) and human enamel on Hawlay retainers in subjects participating in an intraoral research program. They found that in situ caries lesions were developed in both shark and human enamel. Therefore, it seems that the firmly (structurally) bound fluoride may not play a determinant role in the process of inhibiting enamel demineralization.

During the past decades, much attention has been paid to the role of solution fluoride during acid challenge. Arends et al. (39) found that low levels of fluoride in solution could significantly inhibit the dissolution rate of enamel when its surface was
exposed to this media. In the in situ study of Ogaard et al. (38), it was reported that enamel lesion formation was significantly inhibited by the introduction of low levels of fluoride in the demineralization environment (i.e., by giving daily fluoride rinses). An in vitro study conducted by Featherstone et al. (40) demonstrated that initial dissolution rate of carbonated (3% wt) apatite was reduced by fluoride in acetate buffer solutions, and this reduction was proportional to the logarithm of fluoride concentration. In order to directly compare the effect of structurally bound fluoride, adsorbed fluoride, and fluoride in solution, Wong et al. (41) studied the dissolution rate of apatite with various amounts of incorporated or surface-adsorbed fluoride in acetate buffer solutions with or without solution fluoride. They found that a small amount of solution fluoride (0.1 ppm) could have the same effect as a large amount (30,476 ppm) of incorporated fluoride in inhibiting the initial dissolution rate of apatite. The mechanism of loosely bound fluoride (CaF$_2$) in demineralization inhibition was well addressed by Ten Cate et al. (42) based on their series of studies investigating fluoride in solution (between 0 to 10 ppm) and the occurrence of caries. They commented that fluoride would bind calcium and phosphate, forming solid CaF$_2$ when acid solution penetrated into the enamel tissue and dissolved the apatite. This precipitation prevented the mineral content from leaching away into ambient solution and, probably more importantly, the loosely bound fluoride would serve as a reservoir for supplying solution ion fluoride and retarding demineralization. Thus, while both firmly bound fluoride (FAP) and loosely bound fluoride (CaF$_2$) hinder demineralization of enamel apatite, the most effective role to suppress the dissolution of apatites may be the fact that they are potential reservoirs to enhance local ambient solution fluoride concentration.
Using the solubility product of a predetermined stoichiometry, Higuchi et al. (43, 44) have developed a model that can be used to predict the dissolution kinetics of apatites. Using this method, Mir et al. (45) studied powder enamel and synthetic hydroxyapatite and discovered that the demineralization rate was governed by the apparent solubility product (ion activity product) of fluorapatite when acid buffer solutions were spiked with fluoride at concentrations of 1 to 10 ppm. Such results suggested that an FAP surface complex could be central to the mechanism of solution fluoride's influence on the dissolution of apatitic mineral. The proposed FAP surface complex concept was that there would be a rapid exchange of fluoride ions (from the solution) and hydroxide ions (from the solid) on the crystal surface when HAP was exposed to fluoride-containing solutions. It was the new stoichiometry formed on the mineral surface (i.e., FAP), not the solid phase HAP, controlled the dissolution and solubility of the apatite. As a result, enamel and HAP samples possessed the solubility properties of FAP when there was fluoride present in the dissolution media. Experimental results of Wong et al. (41) confirmed the mechanism that F in solution could substitute for OH on the surface of apatite crystal, and that the apatite showed FAP solubility behavior. For the importance of fluoride on the apatite surface, interest has been given to investigating the effect of adsorbed fluoride on the dissolution of apatites. Nelson et al. (46) found that the initial dissolution rates of different fluoride-containing (up to 1000 ppm) apatite samples were not distinguishable in fluoride-free dissolution media while 1 ppm solution fluoride could significantly suppress the demineralization of apatites. They further reported that the adsorption of fluoride on the surface of the apatite crystal was the major factor in the reduction of the dissolution rate. Ludwig et al. (47)
studied the dissolution properties of synthetic CAP, HAP, and enamel in solutions with low levels of fluoride and found that the demineralization rate depended on the solubility product of FAP, not that of CAP or HAP, which was consistent with the proposed mechanism of an FAP surface complex.

In the past 30 years since the beginning of the concept of an FAP surface complex (45), the effect of solution fluoride and adsorbed fluoride on the crystal surface on the dissolution of biological and synthetic apatites have been investigated intensively. However, conclusive results have not been obtained yet. In the work of this dissertation, systematic studies have been performed with the intent to quantitatively understand the functional form (surface complex stoichiometry) governing the solubility behavior of apatites in fluoride-containing media. Also, the pattern of solution fluoride inhibiting CAP solubility (MES) at extremely low levels (the lowest concentrations of solution fluoride which influences the solubility of apatites) has been intensively investigated.

**Fluorine Analysis Methods**

In the past several decades, methods for fluorine (including organic fluorine and inorganic fluoride) analysis have been investigated and many developments have been achieved. For a valid fluoride determination method, it is necessary for the fluoride to be separated from interfering substances, or the disturbance of interfering substances to be effectively suppressed by an appropriate procedure. Also, the concentration of the final sample must be in the right range for a selected method of fluoride measurement. Alternative approaches of fluorine analysis now available were generalized by Venkateswarlu (48, 49) as: potentiometry (fluoride electrode), gas chromatography, molecular/atomic absorption, titrimetry, spectrophotometry, fluorometry, ion
chromatography, mass spectrometry, helium microwave plasma detector / GC, polarography, catalytic methods, enzymatic methods, radioactivation, etc.

Separation of fluoride from interfering substances is the basic requirement of valid fluoride determination in most types of samples. A classic separation method used for many years was distillation of hydrogen fluoride or hydrogen hexafluorosilicate from acid solutions. However, disadvantages of this method were the loss of hydrogen fluoride by reaction with glass vessels and the tendency of contamination of distillates by phosphoric or sulphuric acid (50, 51). When such a method was employed to measure fluoride in soil, plant ash, etc., the final concentration of fluoride in the distillate was found to be decreased because a larger-than-expected volume of solution was collected (49). Venkateswarlu (52) developed a reverse-extraction technique that was able to separate fluoride from interfering substances in a more rapid procedure and obtain a blank with very low levels of fluoride. The reverse-extraction technique required that fluoride in the aqueous phase (containing interfering substances) was first extracted by an immiscible organic phase such as a fluorosilane (RSiF, R represented acyl or aryl group), and then it was reverse-extracted by a small volume (microliters) aqueous phase so that the obtained highly concentrated fluoride solution was free of interfering substances.

One other fluoride separation technique, a diffusion procedure, was originally developed by Singer and Armstrong (53) and later dramatically modified by Taves (54). In this diffusion method, the trapping solution and fluoride sample (containing interfering ions) were placed in the center and outer compartments of a Petri dish, respectively, and fluoride was diffused into the trapping solution with the assistance of hexamethyldisiloxane (HMDS). The author reported that fluoride recovery rates of this
procedure were high (over 90%), and the diffusion time was just several 10 minutes when the diffusion cell was kept agitating at room temperature.

In addition to the techniques which are adequate for the measurement of inorganic fluoride, many efforts have been made to determine organic fluorine. Tsunodi et al. (55) and Chiba et al. (56) utilized an aluminum monofluoride (AlF) molecular absorption method to directly measure total fluoride in orchard leaves, organic compounds, plasma, and urine. It was also reported that the method was reliable and rapid for measuring inorganic fluoride (49). For people exposed to fluorochemicals, fluorine levels in their blood plasma need to be monitored for the industrial hygiene requirement. Oxygen-bomb decomposition techniques (57) were employed to analyze total fluorine and organic fluorine in biological samples. Despite being cumbersome and time-consuming, as claimed by the authors, these methods could yield very reliable results. Based on reductive cleavage of the C-F bond with the sodium biphenyl reagent, Venkateswarlu (58) developed a procedure to convert all covalent fluorine in the sample into inorganic fluoride so that conventional techniques for inorganic fluoride determination could be applied.

For solutions (including water) containing small amounts of interfering ions, fluoride concentration can be analyzed directly by judicious selection of the following techniques: potentiometry (fluoride electrode), titrimetry, spectrophotometry, fluorimetry, aluminum monofluoride (AlF) molecular absorption spectrometry, etc. (48, 49, 55) Among these techniques, while some methods, e.g., AlF molecular absorption spectrometry, measure the total fluoride present in sample, other methods, e.g., fluoride-selective electrode, best detect only free fluoride ions. When complexes of fluoride and
metal ions are formed, appropriate reagents such as citric acid, tartaric acid, EDTA, CDTA, etc. are required to release the fluoride ions from the complexes so that fluoride in the complex forms can be detected by the fluoride-ion-sensitive methods. In many practical situations, fluoride levels in solution samples can be lower than the lowest sensitivity limit of the fluoride analysis technique. In this case, a procedure to concentrate these solution samples is necessary for a valid fluoride determination, which can be accomplished by use of magnesium oxide adsorption (59), calcium phosphate adsorption (60), reverse-extraction (52) or apatite adsorption (61).

Fluoride in hard tissues, e.g., bones and teeth, can be analyzed with either colorimetric reagents following distillation or diffusion of fluoride, or directly with a fluoride-selective electrode (62). For fluoride analysis, bone samples are usually ashed before an appropriate fluoride determination technique is employed. Although good results can be obtained with normal and high fluoride content in bone, the measurement of low-fluoride bone often gives a positive bias result, which is rationalized as the adsorption of extraneous fluoride when bone is ashed. Venkateswarlu (49) reported that such a problem could be overcome by use of reverse-extraction technique, in which fluoride in unashed bone is vigorously extracted through shaking. Recently, fluoride content in biological and synthetic apatites, as well as in acetate buffer solutions, has been determined with a modified diffusion method by Zhuang et al.(2001) (61). Although this method is laborious and time-consuming, many independent experiments have shown that the analytical procedure is reliable and accurate, and the lowest limit of this method can reach 0.02 ppb in a sample with a volume of 2 liters.
References


CHAPTER 3

STATEMENT OF THE PROBLEM

The general objective of this study is to provide a quantitatively mechanistic understanding of solubility properties of carbonated apatites in the presence of solution fluoride. The literature review has shown that there is controversy about the published values of the thermodynamic equilibrium solubility product of hydroxyapatite. Previous work in this laboratory claimed that the thermodynamic equilibrium was not reached for apatites in buffer solutions within the time scale of relevance (days to months). Instead, the solubility behavior of all the biological and synthetic apatites considered thus far could be best explained in terms of metastable equilibrium solubility phenomena. As a result, a primary aim of this work is to find out the adequate base or methodology revealing solubility properties of apatites, and specifically, to investigate if the MES phenomena hold when fluoride is present in the dissolution media.

Upon the fundamental base for the apatite solubility determination, a second aim of this research is to seek the functional form (ion activity product stoichiometry) governing the solubility of apatites in the presence of solution fluoride. When solutions are free of fluoride ions, this laboratory has reported that a surface complex possessing the stoichiometry of hydroxyapatite controls the solubility of apatites. When fluoride is present in solutions, presumably a surface complex with the stoichiometry associated
with fluoride may be the driving force function and this hypothesis will be systematically studied by an appropriate experimental design.

Carbonated apatites have shown a variety of solubilities (MESs) if their carbonate contents or synthesis temperatures are different, and it is generally believed that the solubility of carbonated apatites depends on their crystallinities. The third aim of this study is to establish relationships among solubility, surface complex stoichiometries, and crystallite disorder for carbonated apatites, with the purpose to quantitatively understand the factor(s) determining the solubility of apatites.

For fundamental research interests and applications, the last objective of this work is to investigate the apatite solubility properties at extremely low levels of solution fluoride. This research aims to establish the threshold fluoride concentration that correspond to where fluoride begins to have an inhibitory effect upon carbonated apatite solubility, and to examine the nature of the mechanism of fluoride participation in the solubility reduction. At least two issues have been challenging this study. First, the threshold fluoride concentrations are found to be in the sub-ppb levels, but the lowest limit of the best fluoride analysis technique available can reach only sub-ppm levels. So a procedure has to be developed to determine such low solution fluoride concentrations. The second issue is that, due to the fluoride contamination in the stock chemical reagents (even in analytical grade), fluoride concentration in the dissolution media prepared by these reagents is usually greater than the threshold concentrations affecting the apatite solubility. Therefore, a procedure needs to be developed so that fluoride impurity in the dissolution media can be effectively removed and the solution fluoride levels can be reduced to ultra-low (i.e., < 0.1 ppb).
CHAPTER 4

METASTABLE EQUILIBRIUM SOLUBILITY BEHAVIOR OF CARBONATED APATITES IN THE PRESENCE OF SOLUTION FLUORIDE

Introduction

Epidemiological studies (1) that show an inverse relationship between the fluoride levels in public drinking water and caries incidence have motivated numerous investigations of the role of fluoride in the demineralization and remineralization processes associated with dental enamel. The influence of fluoride on the physicochemical properties of apatites has also been appreciated because of its possible importance in the prevention/treatment of osteoporosis (2-12). Although there have been many attempts in the past, the mechanism of action of fluoride in inhibiting the dissolution tendency of apatites has not been established (13-24). Based on a correlation between the fluoride level of drinking water and the fluoride content of tooth enamel, the cariostatic property of fluoride has been presumed to be related to the formation of a less soluble, fluoride-containing mineral, fluorapatite (FAP) (18, 24). However, some studies have failed to show any significant correlation between the fluoride content of enamel and of carbonated apatites and their dissolution tendencies (20, 25). Another proposed mechanism for fluoride action is based on the role of low levels of solution fluoride on the solubility reduction of apatites (16, 21, 22). It appears that the importance of solution
fluoride in dental enamel dissolution was first demonstrated by Mir et al. (26) who showed that, in the presence of low levels of solution fluoride, the apparent solubility of powdered enamel and a synthetic hydroxyapatite (HAP) preparation was governed by an ion activity product well approximated by that for an entity with the fluorapatite (FAP) stoichiometry. These investigators proposed that an FAP surface complex, formed via an exchange of F for OH ions on the crystal surface, may have accounted for the dissolution retardation. The more recent studies of Wang et al. (27) have provided further support for this hypothesis.

In this report, the influence of solution fluoride on the dissolution tendency of apatites (CAPs) has been determined by applying the concept of metastable equilibrium solubility (MES) distributions. Although the likely importance of MES in the solubility behavior of dental enamel was previously noted by Brown et al. (28), the first quantitative attempt to examine the MES concept was by Hsu et al. (29), who clearly showed that the apparent solubility behavior of the CAPs and human dental enamel is consistent with the MES phenomenon. In 1996 Baig et al. (30) extended the studies of the MES phenomenon for a range of CAPs with varying carbonate content and crystallinities and examined the influence of these variables on the MES behavior of the CAPs. The results of these studies showed that the magnitude of the MES directly correlated with crystallinity and that there was no additional effect of carbonate on the CAP MES once crystallinity was taken into account.

Recently, the MES concept has been extended to the determination of the functional form of the dissolution driving force, which is hypothesized to be associated with surface complex formation on dissolving apatite crystal surfaces (31, 32). It is
envisioned that surface complex formation occurs on the dissolving apatite crystal surface with a composition responsive to the ions in the ambient solution. The results of MES studies in a series of solutions with varying composition have demonstrated that in the absence of solution fluoride the metastable equilibrium solubility (MES) distributions of CAPs are best described by a surface complex possessing the stoichiometry of hydroxyapatite (HAP) (32).

The purpose of the present study was to investigate whether the MES phenomenon holds for the CAPs in the presence of solution fluoride and to test the hypothesis that a surface complex with fluorapatite (FAP) stoichiometry governs the MES of CAPs in the presence of solution fluoride. The application of the MES concept in the determination of the solubility behavior of CAPs in the presence of solution fluoride may provide insight into the mechanism of fluoride action on the dissolution behavior of biominerals (tooth and bone). This could lead to more rational approaches in the development of fluoride delivery systems for the treatment of dental caries and osteoporosis.

**Materials and Methods**

**Carbonated Apatite Synthesis**

Two carbonated apatite preparations were synthesized by the method described by Nelson and Featherstone (33) with some modification. All reagents used were AR grade. The method involved precipitation at 95°C after mixing aqueous solutions of calcium nitrate (Ca(NO₃)₂·4H₂O) and sodium phosphate (NaH₂PO₄·H₂O) at two NaHCO₃ levels. Accurately measured 200 ml of a 0.15 M calcium nitrate solution and 200 ml of a 0.090 M sodium phosphate solution containing 0.0045 and 0.090 M sodium bicarbonate were
added with a peristaltic pump P-3 (Pharmacia Fine Chemicals) at a rate of 1.4 ml/min into 2 liters of doubly deionized water under stirring conditions. The carbonate-to-phosphate molar ratios of the feed were 0.05, and 1. The pH of the mixtures was kept constant at 9.0 (± 0.1) with 1M sodium hydroxide solution using a Radiometer Autoburette type ABU 80 and a titrator type TTT 80. After the addition of the calcium and the phosphate/carbonate solutions was completed, the mixture was digested for one hour. The residue was obtained by filtering the mixture and washing three times with doubly deionized water. The final residue was then freeze-dried and stored in a dessicator for later use.

Physical and Chemical Analysis

The two CAP samples were characterized by x-ray diffraction and infrared analysis. X-ray diffraction data were collected with a Siemens D5000 x-ray diffractometer equipped with a copper target and nickel filter. CuKα rays were generated at 40kV and 20mA. A step size of 0.02° held at 1 sec/step was used for the range of 20 to 90° 2θ. Infrared absorption analysis was performed with a Fourier transform infrared (FTIR) spectrophotometer (Mattson Galaxy 3020); the samples were mixed with KBr and pressed into pellets of 13 mm diameter.

The CAP samples were analyzed for calcium, phosphate and carbonate; calcium by the method of Ray Sarkar and Chauhan (34) and phosphate by the method of Gee et al. (35). The carbonate content of each sample was determined by the microdiffusion method of Conway (36).
Preparation of Buffer Solutions for MES Measurement

A series of 0.1 M acetate buffer solutions was prepared by mixing stock solutions prepared from AR grade CaCl₂ • 2H₂O (Mallinckrodt), NaH₂PO₄ • H₂O (Fisher Scientific) and NaF (Orion Research Inc). The pH of each solution was adjusted to either 4.5 or 5.5 by the addition of concentrated NaOH or HCl and the ionic strength was adjusted to 0.50 M with NaCl. The fluoride concentration was ranged from 0.03 to 12 ppm. The corresponding ion activity products with respect to both FAP and HAP stoichiometries were calculated from the composition of each solution using the program "EQUIL" (MicroMath Scientific Software, Salt Lake City, Utah). The details of the calculations of the driving force functions, K₇₁ and K₉₈, have been reported previously (29, 37).

Determination of Metastable Equilibrium Solubility (MES) Distributions

Details of the procedure for the MES distribution determinations have been presented previously (30, 38). In order to minimize changes in solution composition and the consequent changes in the solution ion activity products during equilibration, a large solution-to-solid ratio has been used in previous studies (31, 32). Accordingly, 10 mg of CAP sample was equilibrated with 2000 ml of buffer solution with stirring (300 rpm) for 48 hours at 30°C. After equilibration, the solutions were filtered and the amounts of the undissolved CAP were determined from the calcium and phosphate analyses of the residue. The fraction of the dissolved CAP was obtained from the difference between the amount of the undissolved residue and the amount of the CAP initially added. The fraction of CAP dissolved in each buffer solution plotted against the solution ion activity product (K₇₁ or K₉₈) gives the MES distribution plots. Here the ion activity products are:
\[
K_{\text{HPO}_4} = a_{\text{Ca}}^{10} a_{\text{HPO}_4}^6 a_{\text{OH}}^2
\]

and

\[
K_{\text{PO}_4} = a_{\text{Ca}}^{10} a_{\text{PO}_4}^6 a_{\text{F}}^2
\]

where the \(a\)'s are the ion activities in the equilibrating solutions.

In experiments where the initial fluoride levels were low in the buffer solutions, the fluoride concentrations of the solution were routinely analyzed after equilibration to make certain that the fluoride levels did not change significantly during equilibration, due to adsorption onto the apatite crystals.

**Results and Discussion**

**Characterization of CAP Samples**

The two CAP samples, now designated here as CAP-1 and CAP-2 were employed in this study. The carbonate contents, analyzed by the Conway's microdiffusion method, were determined to be about 2.1 wt % for CAP-1 and 5.7 wt % for CAP-2, respectively. Figures 4.1(a) and 4.1(b) present the x-ray diffraction patterns for the two CAP samples in a range of 20 to 90° 2θ. As shown in these figures, the x-ray diffraction patterns were characteristic of apatite and solid phases other than apatite were not indicated.

**Determination of Equilibration Time**

The effect of equilibration time on the percent dissolved for CAP-1 at pH 4.5 (closed circle) and pH 5.5 (open circle) are shown in Figure 4.2(a). The solution conditions chosen for these time-dependent experiments were such that approximately 50% of the CAP powder was expected to dissolve. The data in Figure 4.2(a) demonstrate that at each pH the MES plateau was essentially attained after 2 days of equilibration and
Figure 4.1 X-ray diffraction profiles of (a) CAP-1, 2.1 wt% CO$_3$ and (b) CAP-2, 5.7 wt% CO$_3$. 
therefore provided a basis for a reasonable equilibration time to be used for the subsequent MES experiments. Similar experiments have also been done for the effect of equilibrium time on the percent dissolved for CAP-2 at pH 4.5 and 5.5. As can be seen in Figure 4.2(b), the same results as that for CAP-1 have been obtained.

**MES Distributions for CAP-1**

The results of the MES distribution experiments for CAP-1 are presented in Figure 4.3. Here the percent of CAP powder dissolved is plotted against the negative logarithm of the solution ion activity product ($K_{HAP}$) for the hydroxyapatite (HAP) stoichiometry (Figure 4.3(a)) and $K_{FAP}$ for the fluorapatite (FAP) stoichiometry (Figure 4.3(b)). In both sets of plots (i.e., Figures 4.3(a) and 4.3(b)), the experimental data are the same. The pH of the equilibrating solution was 4.5 and 5.5 and the fluoride concentrations were ranged from 0.1 to 5.0 ppm. As shown in these plots, the percent of CAP powder dissolved depended importantly upon the degree of saturation (or unsaturation), represented by $pK_{HAP}$ (Figure 4.3(a)) and $pK_{FAP}$ (Figure 4.3(b)), with greater percentages of CAP dissolving at higher degrees of undersaturation i.e., at larger $pK_{HAP}$ or $pK_{FAP}$ values. These results are consistent with our previous findings (29, 30). These results also demonstrate that the MES distribution phenomenon holds even in the presence of solution fluoride.

The plots shown in Figures 4.3(a) and 4.3(b) now permit us to address the question of whether or not the HAP stoichiometry or the FAP stoichiometry or neither may well represent the stoichiometry of the surface complex governing the MES behavior of CAP-1. Our previous work had shown that in the absence of solution fluoride, a surface complex with the stoichiometry of HAP best described the MES
Figure 4.2 Effect of equilibration time on the dissolution of CAPs. (a) CAP-1 containing 2.1 wt% carbonate at (●) pH 4.5 and (○) pH 5.5. The solution had an ion activity product $K_{\text{FAP}} \sim 10^{-120}$. (b) CAP-2 containing 5.7 wt% carbonate at (●) pH 4.5 and (○) pH 5.5. The solution had an ion activity product $K_{\text{FAP}} \sim 10^{-115.5}$ at pH 4.5 and $10^{-114.5}$ at pH 4.5
Figure 4.3 Cumulative solubility distributions for CAP-l (2.1 wt% carbonate) in different solutions. The percent of CAP powder dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometries of HAP (a) and FAP (b). The same MES data are used in these two sets of plots. For pH 4.5: (○), 5.0 ppm F; (□), 1.0 ppm F; (△), 0.1 ppm F; (◇), 0.03 ppm F. For pH 5.5: (×), 12.0 ppm F; (●), 5.0 ppm F; (■), 1.0 ppm F; (▲), 0.1 ppm F.
behavior of CAPs of varying carbonate contents and crystallinity (32). In those studies, the pH and the solution Ca/P ratio of the equilibrating solutions were used as the two independent variables for deducing the "best" stoichiometry of the surface complex, with pH being more sensitive than the Ca/P ratio in discriminating between the various possible surface complex stoichiometries. In the present study, the pH and the fluoride level of the equilibrating solutions have been varied. For the correct surface complex stoichiometry the experimental MES distributions should be a single valued function of the ion activity product corresponding to the correct surface complex. Figure 4.3(a) shows that, when the surface complex is assumed to have the HAP stoichiometry, there is considerable variability in the positioning of the MES distribution along the abscissa. The MES distributions are seen to shift towards lower solubility values (higher $pK_{\text{HAP}}$) either when the pH of the solution decreased from 5.5 to 4.5 or when the fluoride concentrations in the solution increased from 0.03 to higher values. These displacements of the MES distributions along the abscissa are more sensitive to the changes in the fluoride concentrations of the solution than to the changes in solution pH. As can be seen in Figure 4.3(a), there is a maximum shift in the MES distributions of about 5 in $pK_{\text{HAP}}$ units (compare 0.1 ppm at pH 5.5 with 5.0 ppm at pH 4.5). This magnitude of shift corresponds to about a factor of 2 in terms of molar solubility, which would be greater than experimental variability. This analysis therefore demonstrates that the HAP stoichiometry is not an appropriate model for the surface complex governing the solubility behavior of the CAP-1 in the presence of solution fluoride.

The case where the surface complex with the FAP stoichiometry is assumed is shown in Figure 4.3(b). The MES distributions obtained at the various levels of fluoride
and at the two pH values are much closer to being superimposable here than in Figure 4.3(a). There still remain, however, some systematic variations in the positioning of the MES distributions as a function of solution composition that can be noted in Figure 4.3(b). These variations appear to depend on both the solution pH and on the fluoride levels of the equilibrating solution. When the pH was increased from 4.5 to 5.5 at a given fluoride level or when the fluoride level was decreased at either pH, the MES distributions shifted towards the lower solubility regions of the plot (i.e., to larger $pK_{FAP}$ values). A closer examination of the plots in Figure 4.3(b) reveals, however, an important pattern: at the lower pH of 4.5, the distributions appear to converge to a common location on the abscissa at a fluoride level of around 1.0 ppm; however, at pH=5.5, a higher level of fluoride (~ 12 ppm) is needed to bring the MES distribution plots close to the pH 4.5 convergence limit. Unfortunately, an experiment at a significantly higher level of fluoride at pH 5.5 was not feasible as the solubility product of CaF$_2$ would have been exceeded. These results with CAP-1 have demonstrated, however, that in the limit of low pH and high fluoride levels, the FAP surface complex model may adequately describe the MES behavior of CAP-1.

MES Distributions for CAP-2

Let us now discuss the results obtained with CAP-2. The MES distributions of CAP-2 are presented in Figures 4.4(a) and 4.4(b). As in the case with the CAP-1 results, the abscissa is expressed as the negative logarithm of the solution ion activity product with respect to the stoichiometry of HAP (Figure 4.4(a)) or FAP (Figure 4.4(b)). The pH of the equilibrating solution was again 4.5 and 5.5 and the fluoride concentration was ranged from 0.03 to 5.0 ppm. As was the case with CAP-1 (compare Figures 4.3(a) and
Figure 4.4 Cumulative solubility distributions for CAP-2 (5.7 wt% carbonate) in different solutions. The percent of CAP powder dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometries of HAP (a) and FAP (b). The same MES data are used in these two sets of plots. For pH 4.5: (O), 1.0 ppm F; (□), 0.1 ppm F; (△), 0.03 ppm F. For pH 5.5: (○), 5.0 ppm F; (●), 1.0 ppm F; (■), 0.1 ppm F; (▲), 0.03 ppm F.
4.4(a)), systematic and significant variations of the positions of the MES distribution plot on the abscissa with pH and fluoride concentrations were found for the surface complex based on the stoichiometry of HAP (Figure 4.4(a)). When the surface complex was represented by the stoichiometry of FAP (Figure 4.4(b)), all the MES plots, except for the two low fluoride cases at pH 5.5, can be seen to be essentially superimposable. The MES distribution plots obtained for 0.1 and 0.03 ppm of fluoride at pH 5.5 were slightly displaced towards the right. The results of Figure 4.4(b) are consistent with those of CAP-1 (Figure 4.3(b)), where a surface complex with the stoichiometry of FAP was found to hold quite well under the conditions of the lower pH (4.5) and/or higher solution fluoride concentrations. The CAP-2 results are, however, even more convincing in that the data for the two highest fluoride levels at pH 5.5 have actually essentially converged to the same limit as that seen for pH 4.5.

The findings of this study, which are the first of their kind, can be summed up as follows. First, the present study has importantly shown that the MES distribution is a general phenomenon that can be applied to carbonated apatites over a wide range of solution compositions, including those containing varying levels of solution fluoride. Secondly, a complete superposition of the MES distributions, determined under solution conditions of the lower pH and high fluoride levels, was obtained when the MES data were plotted against the ion activity product based upon the stoichiometry of FAP. However, when the surface complex represented by the HAP stoichiometry was assumed to govern the dissolution of CAP samples, the MES distribution plots exhibited large variations, demonstrating that the HAP stoichiometry poorly represents the MES governing surface complex in the presence of solution fluoride.
Effects of Fluoride in Dental Caries

Because of its role in caries prevention and in the management of osteoporosis, the fluoride ion has been the subject of numerous studies for almost five decades. The mechanism of fluoride action, however, has not yet been clearly elucidated, and at least two theories have been put forward in the past to explain the cariostatic mechanism of fluoride. One theory is based upon the idea that dental enamel becomes more resistant to acid challenges when high levels of fluoride are incorporated into the enamel via the formation of a less soluble fluorapatite or fluorhydroxyapatite phase. The thermodynamic solubility product of fluorapatite has been reported as being smaller than that of HAP (13, 15). However, as Brown et al. (15) have pointed out, these differences in solubility alone caused by the presence of lattice fluoride do not appear to be sufficient to account for the dramatic effects of fluoride in inhibiting dental caries formation. A number of other studies have also indicated that fluoride in the solid phase, even at very high levels, likely has a limited role in the prevention of dental caries. Employing synthetic apatites Nelson et al. (20) demonstrated that CAP initial dissolution rates were indistinguishable from CAPs with three levels of fluoride (up to 1000 ppm) incorporated into the solid phase. However, significant reductions in initial dissolution rates were observed when 1 ppm of fluoride was added to the buffer solutions. Larsen et al. (39) have shown from rat studies that low concentrations of fluoride in the drinking water correlated better with anti-caries effects than structurally incorporated fluoride (about 300-1700 ppm) in dental enamel. The limited importance of fluoride incorporated in the lattice was further demonstrated by Øgaard et al. (25) whose studies showed that, despite having very large amounts of fluoride (~ 32,000 ppm), shark enamel still developed
carious lesions in a human intra-oral model. The findings based upon these and other similar studies has led to the emergence of the second theory that relates the inhibition of apatite dissolution to the presence of solution fluoride.

Although in recent years most researchers (16, 20-23) have come to support the view that fluoride in solution, rather than in the solid, has a dramatic effect on the dissolution tendency of dental enamel, the exact mechanism through which solution fluoride exerts its inhibitory effect has remained controversial. Nelson et al. (20) have suggested that adsorption of fluoride from solution onto crystal surfaces is the primary factor for reducing the initial rates of dissolution when CAP samples are exposed to buffer solutions containing fluoride. Adsorbed fluoride, referred to as “loosely bound fluoride” by Arends et al. (14), has been suggested as the major factor in the prevention of dental enamel caries and, also, in the enamel remineralization process. These investigators have proposed a model consisting of a dynamic equilibrium between fluoride in solution and the crystal surface and have suggested that the dissolution process completely stops when the solution is supersaturated with respect to the fluorapatite phase. Another “dynamic” model proposed by Margolis et al. (19) assumes that the inhibition of dental enamel dissolution by solution fluoride is a result of two competitive processes: demineralization of the HAP phase and remineralization with the FAP phase. This model postulates that under conditions when the solution is undersaturated with respect to HAP and supersaturated with respect to the FAP phase, the increased deposition rate of the FAP phase could effectively inhibit enamel dissolution. The present work, however, advances the view that solution fluoride exerts its effect via the FAP surface complex. The surface complex is considered to be not a
thermodynamically stable phase; rather, it is hypothesized as an entity that forms on a pre-existing template of the dissolving apatite crystal. The composition of the surface complex, as found to possess the stoichiometry of FAP in the present study (at the lower pH and higher fluoride levels), is determined by the ambient ions in the solution. As can be seen in Figure 4.5 (replotted from Figures 4.3(b) and 4.4(b)), the MES values are different for the two CAP samples. Had it been the thermodynamically defined FAP phase controlling the reduction in the MES of the CAPs, as suggested by other investigators, the MES values of both the CAP samples should have corresponded to the solubility product ($K_{sp}$) of the thermodynamically stable FAP phase. If the $K_{sp}$ of the thermodynamically stable FAP phase were to be the solubility governing function, little or no dissolution of the CAP-2 sample would have occurred in solutions corresponding to $pK_{FAP}$ of less than 120, because solutions with $pK_{FAP}$ of less than 120 would be supersaturated with respect to the thermodynamically stable FAP phase ($pK_{sp}$ of FAP has been reported to be around 120.3) (13, 15). On the contrary, the two CAP samples yielded different values for the mean of the MES distributions; mean $pK_{FAP} = 119.8$ for CAP-1 and $pK_{FAP} = 115.4$ for CAP-2. Based upon our previous studies (30) on the relationship between the mean MES of the CAP samples and crystallinity, the present results are consistent with the interpretation that, even though the functional form of the MES is best represented by the surface complex based upon FAP stoichiometry, the magnitude of the MES is determined by the crystallinity of the CAPs.

The idea of an FAP surface complex governing the apparent solubility of dental enamel was first suggested by Mir et al. (26), who demonstrated that in the presence of low fluoride levels in the buffer solution, the apparent solubility of enamel and HAP can
Figure 4.5 Comparison of the cumulative solubility distribution of CAP-1 and CAP-2, respectively by the surface complex based upon the stoichiometry of FAP. Data were selected from Figures 4.3 and 4.4. The symbols are the same as in Figures 4.3 and 4.4.
be best described by the ion activity product based on the stoichiometry of FAP. This FAP surface complex was proposed to be due to the exchange of F\textsuperscript{−} with OH\textsuperscript{−}, forming on a template of the dissolving apatite crystal surface and mimicking the composition of the thermodynamically stable FAP phase. However, those studies were based upon dissolution kinetics experiments and lacked good sensitivity to the quantitative determination of the form of the driving force function for dissolution. The present method, using the MES concept and a range of solution compositions, is much more sensitive in the determination of the surface complex composition. The results of the present study have consequently provided important support that, in the presence of solution fluoride, a surface complex that assumes the stoichiometry of FAP governs CAP dissolution.

Conclusions

The investigation of the solubility behavior of two carbonated apatite preparations has demonstrated that the MES concept is generally applicable over wide range of pH and solution fluoride concentrations. Additionally, the surface complex based upon the stoichiometry of HAP, previously found to best describe the solubility behavior of CAPs in solutions containing only calcium and phosphate (no fluoride), was found not to be an appropriate model when the MES data were obtained in the presence of solution fluoride. On the contrary, the results of the present study indicate strong support for the view that the MES governing surface complex assumes the stoichiometry of FAP in the presence of solution fluoride. An FAP surface complex controlled limiting case was found to hold for the MES data obtained at the lower pH of 4.5 and for the higher levels of fluoride in solution. A slight, but systematic deviation from this FAP controlled limiting case was
observed when the pH of the solution was increased and/or fluoride concentrations were decreased.

The findings of the present study provide a new insight into the mechanistic understanding of the role of fluoride in reducing the dissolution tendency of apatites. These results could contribute toward better approaches for the design of therapeutic regimens for fluoride delivery.

Appendix

Studies have shown that the FAP surface complex model can best describe the MES behavior of CAP-1 and CAP-2 in the limit of low pH and high fluoride levels (e.g., pH 4.5 and 1.0 ppm F). Further investigations on the CAPs solubility distributions at pH 3.5 and 1.0 ppm F have been done and the results are shown in Figure 4.6. It is evident that a surface complex with the stoichiometry of FAP governs the CAP MES behavior for both the two CAP samples when solutions pH and fluoride concentration are of 3.5 and 1.0 ppm, respectively. Therefore, this in depth study further supports the main conclusions of this research project.
Figure 4.6 The cumulative solubility distribution of CAP-1 and CAP-2 in solutions of pH 3.5 and 1.0 ppm F (+) by the surface complex based upon the stoichiometry of FAP. The other data were selected from Figures 4.3 and 4.4, and the symbols are the same as in Figures 4.3 and 4.4.
References

CHAPTER 5

RELATIONSHIPS INVOLVING METASTABLE EQUILIBRIUM
SOLUBILITY, SURFACE COMPLEXES, AND
CRYSTALLINITY DISORDER WITH
CARBONATED APATITES

Introduction

Carbonated apatites (CAPs) have been the focus of numerous investigation for the last two decades because they have served as an excellent model system for studying the physicochemical properties of biological hard tissues (1-4). In 1994 Hsu et al. (5) reported that CAPs and human dental enamel exhibit the phenomenon of metastable equilibrium solubility (MES) and that there is a distribution of MESs for a given apatite preparation. The MES concept has been shown to apply to all apatite preparations investigated thus far, including CAPs prepared at different temperatures of synthesis with varying carbonate contents and crystallinities (3, 6) and bone mineral from rats of different ages (7). The results of these studies have shown that the magnitude of the MES directly correlates with crystallinity and that there appears to be no additional effect of carbonate on the CAP MES once crystallinity is taken into account.

Recently, the MES concept was extended to the determination of the functional form of the dissolution driving force, which is hypothesized to be associated with surface
complex formation on dissolving apatite crystal surfaces (7, 8). It has been envisioned that surface complex formation occurs on the dissolving apatite crystal surface with a surface complex composition responsive to the ions in the ambient solution. The results of MES studies in a series of solutions with varying composition have demonstrated that the metastable equilibrium solubility (MES) distributions of CAPs are best described by a surface complex with the hydroxyapatite (HAP) stoichiometry in the total absence of solution fluoride (8) and by a surface complex with the fluorapatite (FAP) stoichiometry when appreciable solution fluoride is present (9). These studies have also demonstrated that, while the composition of the MES governing surface complex is influenced by the composition of the solution, the magnitude of the dissolution driving force (i.e., the apparent solubility) appears to be primarily determined by the preexisting state of the mineral or mineral domains. An important question then arises as to how the preexisting energetics of the CAP crystal domains are linked to the composition and energetics of the MES governing surface complexes.

A main purpose of the present study was then to address this question by determining the effect of the change in the surface complex stoichiometry (from that of HAP to that of FAP) upon the relationship of the MES to crystallinity. Another purpose of this study was to examine the hypothesis that, although the dissolution driving force function for the CAPs has the same functional form as that of the thermodynamically stable hydroxyapatite (in the absence of solution fluoride) and fluorapatite (in the presence of solution fluoride) phases, they cannot be associated with the solubility product of the thermodynamically stable HAP and FAP phases.
Materials and Methods

Carbonated Apatite Synthesis

Carbonated apatites (CAPs) were synthesized using two different procedures (Method A and Method B) which have been described previously. Method A (3) basically involves CAP synthesis by precipitation from calcium nitrate and sodium phosphate (containing sodium bicarbonate) solutions at constant pH at temperatures of 70, 85, and 95°C. In Method B (6), CAPs were synthesized employing a variation of the procedure of LeGeros et al. (10) involving the hydrolysis of dicalcium phosphate dihydrate (DCPD) for 48 hours in sodium bicarbonate containing media at temperatures of 50, 70, and 95°C.

X-ray Diffraction Analysis

In the case of CAPs synthesized by Method A, X-ray diffraction data were collected for analysis by the Rietveld method of whole-pattern-fitting structure-refinement. Figure 5.1 (a) shows the X-ray diffraction pattern of CAP 702 (see Table 5.1), which is the representative for the CAPs synthesized by Method A. The procedures for the X-ray diffraction experiments and the Rietveld method for simultaneously determining the crystallite size and microstrain parameters have been previously described in detail (3).

X-ray diffraction experiments with the CAPs synthesized by Method B were performed on a Siemens D5000 x-ray diffractometer equipped with a copper target and a nickel filter. X-rays were generated at 40 kV and 20 mA. A step size of 0.05° held for 0.3 s/step was used for the range of 20° to 40° 2θ. A detailed scan of 002 reflection, ranging from 24 to 27.5° 2θ was carried out using a step size of 0.01° and held for 5 s/step. By use of the computer program PRO-FIT (Toraya 1986 (11)) the full width at half maximum
Table 5.1. Chemical composition of carbonated apatite samples prepared by Method A.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Synthesis Temperature (°C)</th>
<th>CO$_3$ (wt%)</th>
<th>PO$_4$ (wt%)</th>
<th>Ca (wt%)</th>
<th>Microstrain (X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>702</td>
<td>70</td>
<td>3.0</td>
<td>47.9</td>
<td>37.2</td>
<td>0.3459</td>
</tr>
<tr>
<td>852</td>
<td>85</td>
<td>2.2</td>
<td>50.9</td>
<td>36.8</td>
<td>0.2422</td>
</tr>
<tr>
<td>853</td>
<td>85</td>
<td>3.9</td>
<td>51.1</td>
<td>36.9</td>
<td>0.3749</td>
</tr>
<tr>
<td>854</td>
<td>85</td>
<td>4.4</td>
<td>48.4</td>
<td>36.1</td>
<td>0.4407</td>
</tr>
<tr>
<td>951</td>
<td>95</td>
<td>1.9</td>
<td>47.7</td>
<td>39.8</td>
<td>0.0932</td>
</tr>
<tr>
<td>953</td>
<td>95</td>
<td>6.0</td>
<td>48.7</td>
<td>38.4</td>
<td>0.2841</td>
</tr>
</tbody>
</table>
(FWHM) of this reflection was determined for each sample and then used as an indicator of crystallinity (crystallite size/disorder). Figure 5.1 (b) demonstrates the X-ray diffraction profiles of CAP 102, 103 and 104 (see Table 5.2), which are the representatives for the CAP samples synthesized by Method B.

Chemical Analytical Methods

The CAP preparations were analyzed for calcium, phosphate and carbonate. Calcium concentrations were determined by the method of Ray Sarkar and Chauhan (12) and the phosphate concentrations by the method of Gee et al. (13). The carbonate content of each sample was analyzed by the microdiffusion method of Conway (14) and the method of Taves (15) was employed for the determination of fluoride concentrations.

Metastable Equilibrium Solubility Determinations

MES distributions of the CAP preparations were determined by a previously described method (5, 6, 8, 9). Briefly, the method involves exposing small amounts of powdered CAP samples to a series of 0.1 M acetate buffer solutions containing various levels of calcium, phosphate and fluoride ions. The solutions were prepared by mixing calculated amounts of AR grade CaCl₂ (Mallinckrodt), NaH₂PO₄ (Fisher Scientific) and NaF (Orion Research) stock solutions. The pH of 4.50 ± 0.01 of each solution was attained by the addition of NaOH, and NaCl was used to adjust the final ionic strength of all MES solutions to 0.50 M. A fluoride concentration of 0.10, 1.0, or 5.0 ppm was employed in the MES determinations of all CAP samples. Ion activity products (K_{HAP}, K_{FAP} and/or K_{FHAP}) were calculated from the composition of each solution using the program "EQUIL" (MicroMath Scientific Software, Salt Lake City, UT)(8). It is
Figure 5.1 X-ray diffraction patterns of CAPs. (a) CAP 702 synthesized by Method A (see Table 5.1). (b) CAP 102, 103 and 104 synthesized by Method B (see Table 5.2). The numbers in parentheses are Miller indices.
Table 5.2. Chemical composition of carbonated apatite samples prepared by Method B.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Synthesis Temperature (°C)</th>
<th>CO\textsubscript{3} (wt%)</th>
<th>PO\textsubscript{4} (wt%)</th>
<th>Ca (wt%)</th>
<th>FWHM (°2θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>95</td>
<td>1.0</td>
<td>54.9</td>
<td>34.1</td>
<td>0.3601 (0.0017)\textsuperscript{a}</td>
</tr>
<tr>
<td>103</td>
<td>95</td>
<td>3.9</td>
<td>51.4</td>
<td>35.9</td>
<td>0.4052 (0.0010)</td>
</tr>
<tr>
<td>104</td>
<td>95</td>
<td>6.2</td>
<td>49.6</td>
<td>35.4</td>
<td>0.4325 (0.0004)</td>
</tr>
<tr>
<td>106</td>
<td>70</td>
<td>1.8</td>
<td>54.4</td>
<td>35.0</td>
<td>0.4251 (0.0025)</td>
</tr>
<tr>
<td>110</td>
<td>50</td>
<td>1.3</td>
<td>53.3</td>
<td>33.6</td>
<td>0.4470 (0.0076)</td>
</tr>
<tr>
<td>111</td>
<td>50</td>
<td>3.5</td>
<td>51.2</td>
<td>33.9</td>
<td>0.4877 (0.0059)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The values within parentheses are standard deviations of duplicate runs.
important to note that for an accurate quantification of the MES, the solution composition must not change significantly during the equilibration run. Accordingly, a large solution to solid ratio (2000ml/10mg) was employed in the present study. In order to make certain that the composition did not significantly change during equilibration, the solutions were analyzed for calcium, phosphate and fluoride both before and after equilibration. The CAP preparations were equilibrated with stirring (300 rpm) for 48 hours at 30°C. After equilibration, the undissolved CAP residues were recovered by filtration and quantitated, and the fractions of CAP powder dissolved in buffer solutions with different compositions were plotted against the solution ion activity product of interest.

Results and Discussion

Carbonated Apatite Samples in this Study

Table 5.1 summarizes the chemical composition and crystallinity data of the CAP samples in this study prepared by the precipitation method (Method A). Table 5.2 gives the data for the samples used in this study prepared by Method B. Both the CAP samples in Table 5.1 and those in Table 5.2 were from larger groups of samples investigated in earlier studies (3, 6) in which more extensive characterizations of the CAPs were carried out. The selections for Table 5.1 and Table 5.2 were made on the basis of having enough representative CAP samples over essentially the full ranges of crystallinities previously determined.
Relationship between the Solubility (MES) of the CAP Samples of Table 5.1 and the Crystallite Microstrain Parameter (X)

Let us first examine the question of whether the FAP stoichiometry, Ca_{10}(PO_4)_6F_2, is the appropriate MES governing function when sufficiently high levels of solution fluoride are present for those CAP samples prepared by Method A (Table 5.1). It was established recently (8) that, in the absence of solution fluoride, the MES behavior of the CAPs is well described by the surface complex possessing the stoichiometry of HAP, Ca_{10}(PO_4)_6(OH)_2. It was also established in another recent study (9) that the MES behavior of CAPs in the presence of solution fluoride may be best represented by the FAP [Ca_{10}(PO_4)_6F_2] surface complex when the solution fluoride concentrations were sufficiently high.

The present results of the MES distribution experiments with the six CAPs of Table 5.1 are shown in Figures 5.2 - 5.7. For each CAP sample the experiments were conducted at two solution fluoride levels (1.0 and 5.0 ppm F for samples 951 and 852; 0.10 and 1.0 ppm F for samples 953, 702, 853, 854). The CAP percent dissolved values are plotted against the ion activity products for HAP [Ca_{10}(PO_4)_6(OH)_2], for FHAP [Ca_{10}(PO_4)_6F(OH)], and for FAP [Ca_{10}(PO_4)_6F_2]. It can be readily seen that there is poor superpositioning of the MES distribution data for the HAP and the FHAP stoichiometries but rather good superpositioning of the MES distributions for the FAP stoichiometry. This finding is in good agreement with the earlier study of Zhuang et al. (9) and supports the view that in the presence of sufficiently high levels of solution fluoride, the CAP MES governing entity (i.e., the surface complex) possesses the FAP stoichiometry.
Figure 5.2 Cumulative solubility distributions for CAP 951 synthesized by Method A (see Table 5.1). The percent CAP powder dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometry of HAP, FHAP and FAP, respectively. The curves are obtained by fitting the data to equation \( y = 50 \times \{1 + \text{erf}[k(x-x_0)]\} \), where erf stands for error function (an integral of normal distribution function). Partially saturated buffer solutions are of different fluoride levels (O) 1.0 ppm, and (x) 5.0 ppm.
Figure 5.3 The percent CAP 852 (see Table 5.1) dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometry of HAP, FHAP and FAP, respectively. Partially saturated buffer solutions are of different fluoride levels (O) 1.0 ppm, and (×) 5.0 ppm. (Caption of Figure 5.2 gives more details.)
Figure 5.4 The percent CAP 953 (see Table 5.1) dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometry of HAP, FHAP and FAP, respectively. Partially saturated buffer solutions are of different fluoride levels (■) 0.1 ppm, (O) 1.0 ppm. (Caption of Figure 5.2 gives more details.)
Figure 5.5 The percent CAP 702 (see Table 5.1) dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometry of HAP, FHAP and FAP, respectively. Partially saturated buffer solutions are of different fluoride levels (■) 0.1 ppm, (O) 1.0 ppm. (Caption of Figure 5.2 gives more details.)
Figure 5.6 The percent CAP 853 (see Table 5.1) dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometry of HAP, FHAP and FAP, respectively. Partially saturated buffer solutions are of different fluoride levels (■) 0.1 ppm, (O) 1.0 ppm. (Caption of Figure 5.2 gives more details.)
Figure 5.7 The percent CAP 854 (see Table 5.1) dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometry of HAP, FHAP and FAP, respectively. Partially saturated buffer solutions are of different fluoride levels (■) 0.1 ppm, (O) 1.0 ppm. (Caption of Figure 5.2 gives more details.)
Let us now address the issue of the relationship of the CAP MES to the crystallite microstrain parameter (\(X\)). In this connection, it is both convenient and instructive to plot the mean pK\(_{\text{FAP}}\) values taken from Figures 5.2 – 5.7 against the microstrain parameter. This plot is shown in Figure 5.8. For comparison, the experimental mean pK\(_{\text{HAP}}\) values obtained previously (3, 8) from MES distribution experiments conducted in the absence of solution fluoride are also presented (close circles). Within the data scatter, the MES data obtained in the presence of solution fluoride and represented by the mean pK\(_{\text{FAP}}\) essentially lie parallel to the MES data obtained in the absence of solution fluoride and represented by the mean pK\(_{\text{HAP}}\). It seems reasonable to conclude from this that, while the MES governing entity (i.e., the surface complex) has changed from that with a stoichiometry of HAP (when solution fluoride is absent) to that with the FAP stoichiometry (in the presence of solution fluoride), the energetics of the two surface complexes reflect in a similar fashion the energetics of the pre-existing state of the CAP as expressed by its microstrain parameter value. To expand upon this, it can be said that the differences in crystallite disorder from one CAP sample to another affects the energies of the two surface complexes (HAP and FAP) essentially to the same extent.

Analysis of Results of the Solubility (MES) Experiments with CAP Samples of Table 5.2

The CAP samples of Table 5.2 were synthesized (Method B) (as mentioned earlier) employing the procedure involving the hydrolysis of dicalcium phosphate dihydrate and their crystallinity characteristics have been described previously (6). These preparations could not be subjected to the Rietveld analysis of microstrain and crystallite
Figure 5.8 The relationship between the mean MES values (i.e., mean $pK_{\text{HAP}}$) and the crystallinity parameter (microstrain) when solution fluoride is absent (HAP surface complex, ●) and when solution fluoride is present (FAP surface complex: Δ, 0.1 ppm F; □, 1.0 ppm F; O, 5.0 ppm F). The CAP samples investigated are shown in Table 5.1.
size separation because of fewer and overlapping X-ray diffraction peaks, especially at higher 2θ values. Consequently, the full width at half maximum (FWHM) of the 002 peak has been used here as a measure of crystallinity (6) for these CAP preparations.

Let us now examine the effect of crystallinity on the MES distributions of CAPs prepared by Method B. Here, similar to the data treatment for CAPs prepared by Method A (Figure 5.8), the mean pK\textsubscript{FAP} values determined by fitting the MES data to an error function (an integral of normal distribution function, see Figure 5.9) are plotted against the crystallinity parameter, FWHM, and the result is shown in Figure 5.10. As in Figure 5.10, two sets of experimental data are shown here; one set represents the MES data obtained previously (6) in buffer solutions containing no fluoride and the other (MES data from the present study) from solutions containing fluoride at a level of 1.0 ppm which was deemed to be sufficiently high, based on several test experiments, that the surface complex stoichiometry governing MES would be that of FAP. As can be seen in Figure 5.10, both sets of data are linearly related to the crystallinity parameter and, as was the case for the Table 5.1 CAP results (shown in Figure 5.8), the lines for the two data sets are essentially parallel. Furthermore, it is noteworthy that the difference between the pK\textsubscript{FAP} and the pK\textsubscript{HAP} values are (for any data pair) in the same range of 2 to 3 pK\textsubscript{HAP} units for both the Figure 5.8 and Figure 5.10 results. These findings (Figure 5.10) provide a significant degree of generalization of the conclusion based on the Figure 5.8 results regarding the relationship between the HAP surface complex and the FAP surface complex and their relationship to the crystallinity of the CAP phase.
Figure 5.9 Cumulative solubility distributions for CAPs synthesized by Method B. The percent of CAP powder dissolved is plotted against the negative logarithm of solution ion activity product based upon the stoichiometry of FAP. The symbols are CAP 102 (O), 103 (●), 104 (□), 106 (■), 110 (Δ), and 111 (▲) (see Table 5.2). The curves are obtained by fitting the data to equation \( y = 50\{1 + \text{erf}(k(x-x0))\} \), where \( \text{erf} \) stands for error function.
Figure 5.10 The relationship between the mean MES values (i.e., mean pK_{\text{IAP}}) and the crystallinity parameter (FWHM) when solution fluoride is absent (HAP surface complex, ●) and when solution fluoride is present (FAP surface complex, □). The CAP samples investigated are shown in Table 5.2.
Role of Fluoride in the Prevention of Dental Caries

The role of fluoride in the prevention of caries process has not, to this day, been clearly understood. Although it is generally accepted that fluoride acts through a physicochemical mechanism (i.e., enamel solubility reduction), the exact mechanism of fluoride action in reducing the dissolution tendency of apatites is not known. Some studies have suggested that the reduction in enamel solubility in the presence of fluoride is due to the incorporation of fluoride in the enamel crystal lattice resulting in the formation of a relatively less soluble fluorapatite (FAP) phase (16, 17). Based on this, it has been proposed that the dissolution of enamel crystallites completely stops when the solution is saturated with respect to the FAP phase (18, 19). Other studies, however, have failed to show any significant correlation between the fluoride content of the enamel and of CAPs and their dissolution tendencies (1, 20). This has led to an alternative view that fluoride in solution, rather than in the solid, has the principal inhibiting effect on the dissolution tendency of dental enamel (21-23). The importance of solution fluoride in dental enamel dissolution was first proposed by Mir et al. (24), who showed that, in the presence of low levels of solution fluoride, the apparent solubility of powdered enamel and synthetic hydroxyapatite (HAP) could be associated with an entity possessing the stoichiometry of FAP. These investigators proposed a surface complex model where a surface complex possessing the FAP stoichiometry is formed via an exchange of F⁻ for OH⁻ at the dissolving apatite crystal surfaces. The surface complex has been viewed as an entity that forms on a preexisting template of the dissolving apatite crystal with its composition determined by the ambient ions in the solution.
Recently, Zhuang et al. (9) have provided further support for the FAP surface complex hypothesis in their study of the influence of solution fluoride on the MES behavior of two CAP preparations. Zhuang et al. (9), by employing the MES concept, showed that in the limit of low pH ($\lesssim 4.5$) and sufficiently high solution fluoride concentration, the best function describing the MES behavior of CAPs is that represented by a FAP surface complex. An extensive range of fluoride concentrations and pH were employed in that study; when the MES data were plotted against the solution ion activity product based upon the FAP stoichiometry, a good superpositioning of the MES distribution data as a function of solution pH and fluoride concentrations was obtained, while such was not the case when the MES data were plotted against the ion activity product based upon the HAP stoichiometry.

That the apparent solubility of CAPs is governed by the FAP surface complex and not the thermodynamically stable FAP phase can be easily understood from the results of Figure 5.8 and Figure 5.10. If the fluoride effect were to be the result of the formation of the thermodynamically defined fluorapatite phase, the mean MES values of all of the CAP samples should have been the same and having a $K_{\text{FAP}}$ value of around $10^{-121}$ to $10^{-120}$ as reported by Moreno et al. (25) and by McCann (26) for the solubility product ($K_{\text{sp}}$) of the thermodynamically defined FAP phase. As can be seen, however, in Figures 5.8 and 5.10, the mean $K_{\text{FAP}}$ values are different for the different CAP samples. This supports the present view that surface complexes, rather than the thermodynamically stable FAP or HAP phases, govern the solubility behavior of the CAPs.
Unified View of the Interrelationships Involving MES, Surface Complexes, and Crystallite Microstrain (Disorder)

All CAPs investigated to date (3, 5-9) possess a distribution of MESs. A MES distribution for a CAP sample is a consequence of there being a distribution of crystallite domains having different free energies arising from different degrees of domain disorder. CAP microstrain is a measurable (X-ray diffraction) property which, for a given CAP sample, is a measure of a mean degree of crystallite disorder. It would therefore not be surprising to find the correlation (as was found; see Figures 5.8 and 5.10) between the mean MES (i.e., mean $pK_{\text{HAP}}$) and the experimental microstrain (crystallinity). A CAP sample with a high degree of average lattice disorder should have a high microstrain value and a high mean MES value. To our knowledge, such a significant relationship between crystallite solubility and crystallite microstrain (as seen in Figure 5.8) has never previously been demonstrated experimentally, for any substance. A single phase of a pure compound can possess only one solubility value at a given temperature and pressure (the Ksp principle). However, if the phase in question possesses varying degrees of crystallite (or crystallite domain) disorder or defects and if recrystallization of the thermodynamically stable phase is negligible during equilibration in the MES solution, it would seem quite reasonable to expect variable solubility. Here it would be worthwhile to mention that Patel and Brown (27) concluded that “the most probable cause of the variable solubility product of enamel mineral is the presence of varying amounts of structural defects and impurities in the enamel crystals” (present author’s italics).

Let us now examine the nature of the surface complex and its relationship to the aforementioned disordered crystallite domains. The crystallite domains of CAPs are
basically apatitic (as revealed by X-ray diffraction). When such domains are equilibrated in solutions that are not undersaturated with respect to the domain MES, it would seem reasonable that the basic apatitic architecture can be sustained and that the exchange of phosphate (in solution) -for-carbonate (at the surface of the equilibrating domains) may occur rapidly and essentially completely if the solution phosphate/carbonate is very high. Thus, it is postulated that this phosphate-for-carbonate exchange may take place in or at the surface of the domains in question and the resulting entity would possess the composition of HAP and not that of the original CAP sample itself. With this line of reasoning we can arrive at the conclusion that the MES may be controlled by a function $K_{HAP}$ associated with a “phase” (actually, the surface complex) with the HAP stoichiometry, but the solubility itself can vary because the degree of disorder of the crystallite domains of the “phase” can vary.

Let us now examine what may take place when the equilibrating MES solution contains a relatively high level of fluoride (i.e., solution $F^\to >>$ solution $OH^\to$). Analogous to the phosphate-for-carbonate exchange discussed in the previous paragraph, we postulate that, in addition to the phosphate-for-carbonate exchange, $2F^\to$ for $2OH^\to$ exchange may take place in or at the surface of the domain but without any significant disturbance of the basic apatitic architecture of the domain, this permitting the basic energetics of the domain to remain intact when the $F^\to$ for $OH^\to$ exchange takes place. The $F^\to$ for $OH^\to$ exchange results in the formation of a new surface complex that possesses the FAP stoichiometry. Perhaps most remarkable in all of this is that the new FAP surface complex and the corresponding HAP surface complex both have “inherited” the energetics of the pre-existing CAP domains. The crystallite microstrain of the parent CAP is
reflected in both the mean $pK_{\text{HAP}}$ and the mean $pK_{\text{FAP}}$ (Figures 5.8 and 5.10). The "template" for both the HAP and the FAP surface complexes appears to be the same: it is apatitic, it permits phosphate-for-carbonate and fluoride-for-hydroxide ion exchanges, and its energetics is transmitted to both surface complexes to the same extent (i.e., parallel lines in Figures 5.8 and 5.10).

Conclusions

The metastable equilibrium solubility behavior of carbonate apatite preparations of varying crystallinities was determined in the presence of solution fluoride. The results of this investigation have, for the first time, provided conclusive data that supports the view that solution fluoride acts through a surface complex mechanism and not by forming a surface layer of a separate thermodynamically stable FAP phase. Additionally, the plot of the mean MES (i.e., mean $pK_{\text{FAP}}$) values versus the crystallinity parameter (i.e., crystallite microstrain or FWHM) yielded a linear relationship with a slope essentially the same as that obtained in the absence of solution fluoride (i.e., when the mean $pK_{\text{HAP}}$ values were plotted against crystallite microstrain or FWHM). This parallel situation relating the MES to crystallinity when solution fluoride is present (i.e., FAP surface complex) and when solution fluoride is absent (i.e., HAP surface complex) has taken the physical meaning of the MES governing surface complex to a new level. The degree of the CAP crystallite disorder affects the energetics of both surface complexes (FAP and HAP) essentially to the same extent over a wide range of crystallinities, i.e., the energetics of the pre-existing CAP crystallite domains are "inherited" by both surface complexes to the same extent.
References


CHAPTER 6

EFFECT OF TRACE-LEVEL SOLUTION FLUORIDE
ON SOLUBILITY BEHAVIOR OF
CARBONATED APATITE

Introduction

The inhibitory effect of fluoride on the dissolution of dental minerals has been intensively investigated (1-7). Although some results suggested that fluoride-containing apatites have lower solubilities compared to apatites without fluoride (8, 9), most results have shown, as Brown et al. (10) pointed out that the reduction of solubility resulting from incorporation of fluoride is not sufficient to account for the dramatic effect of fluoride on its caries-preventive role. These results showed that solution fluoride could significantly reduce dissolution rate of apatites, even if the fluoride levels in solutions were low. Although several reasons have been used to describe the inhibitory effect, up to now there is no mechanism that has been widely accepted. Some researchers have demonstrated that the inhibition of dissolution is because of the reduction of the solubility of dental minerals by solution fluoride (1, 2, 11). Mir et al. (2) reported that low levels of solution fluoride can reduce the solubility of apatites. This solubility behavior in the presence of solution fluoride was believed abiding $I_{FAP}$, i.e., the solution ion activity product with respect to fluorapatite stoichiometry (FAP, $Ca_{10}(PO_4)_6F_2$). A surface
complex hypothesis was suggested to explain the phenomenon. The recent studies in our laboratory also supported this hypothesis (11). This assumption is that an FAP surface complex is formed during dissolution by the \textit{in situ} ion exchange of free F$^-$ at the surface of the dissolving crystals. This FAP surface complex can make dental mineral act as FAP-like crystals, even when the uptake amount of fluoride on the crystals is low, and controls the solubility of dissolving mineral by an ion activity product with respect to FAP stoichiometry ($I_{\text{FAP}}$). However, the value of the ion activity product is not the same as the solubility product of the thermodynamically stable FAP phase, instead, it varies and depends on the crystallinity of the mineral. A similar viewpoint was also presented by Featherstone \textit{et al.} (1)

The solution fluoride concentrations involved in most of the current studies (including the studies mentioned above) are ppm (parts per million, 1 ppm = 5.26×10$^{-5}$ M) or higher. There has been no report on the effects of solution fluoride in ppb (parts per billion, 1 ppb = 5.26×10$^{-8}$ M) or even lower levels. Although some reports have suggested that the demineralization of dental enamel blocks is not affected by about 10 ppb of solution fluoride (12), this value is actually the initial concentration of solution fluoride. At such low fluoride concentrations, most of fluoride initially in solution might have been adsorbed during dissolution, and the residual concentration of free solution fluoride in the region close to the surface of solid would be considerably lower. Therefore, the conclusions that the demineralization of tooth enamel would not be interfered by 10 ppb of fluoride may not be valid in other dissolution conditions.

According to the surface complex hypothesis, free solution fluoride concentration as low as 1 ppb may have an inhibitory effect on apatite dissolution. For example, a
partially saturated weakly acid solution with ion activity product (IAP) in terms of HAP stoichiometry (i.e., $I_{\text{HAP}}$) $10^{-118}$ and pH 4.5 may be undersaturated with respect to some apatite preparations. However, if this solution is contaminated by 1 ppb free solution fluoride, its $I_{\text{AP}}$ becomes about $10^{-114}$. Therefore, the fluoride-contaminated solution may be saturated with respect to these apatite samples. Without knowing the contamination, it is easy to draw a wrong conclusion that these apatite samples are not soluble in the solution of $I_{\text{HAP}} 10^{-118}$.

The major obstacle of the research on fluoride’s effect at trace levels is the lack of an appropriate analytical technique for the determination of solution fluoride at such low levels. Currently, the most often used technique is the fluoride-selective electrode. The lower limit of detection of the electrode is claimed by the manufacturer as 0.02 ppm, or 20 ppb (e.g., Radiometer America Inc., type FK1505F).

Lack of a sensitive analytical technique often causes researchers to neglect the effect of free solution fluoride at trace levels. Many reagents, even analytical grade, contain fluoride impurity. Dissolution media made by these reagents will therefore contain trace-level fluoride contamination. During dissolution, part of the fluoride may be adsorbed by the dissolving or nondissolving mineral surface. The concentration of free solution fluoride depends largely on the ratio of the amount of solid and the volume of solution (hereafter referred as solid-to-solution ratio) of the system and the surface area of the mineral. The concentration of free fluoride may even increase during dissolution as the surface area of the dissolving solid decreases and the adsorbed fluoride is released back to the solution. Therefore, without measuring the free solution fluoride, the inhibitory effect
of trace-level fluoride becomes unpredictable, and in some cases, the extent of the effect may even vary with time.

On the other hand, it was recently found in our laboratory (13) that dental enamel and CAPs exhibited a metastable equilibrium solubility (MES) distribution behavior. This behavior is currently under investigation in various solution conditions. Our preliminary results showed that the fluoride impurity present in reagents significantly affects the solubility results of CAPs. Therefore, it was the intention of this study: 1) to establish and evaluate a defluoridation procedure so that the solubility behavior of CAPs can be assessed without fluoride interference, 2) to establish a technique to determine the concentrations of solution fluoride at trace levels, 3) with the help of these powerful tools, to examine the threshold of the fluoride inhibitory effect, and to determine the effects of trace-level fluoride on the solubility behavior of CAP.

Materials and Methods

Removing Fluoride from Solutions (Defluoridation)

Many reagents involved in this study contain trace levels of fluoride. To remove the fluoride impurity from the solutions made by these reagents, a special de-fluoridation procedure was applied to these solutions. In the defluoridation procedure, commercial Bio-Gel HAP powder (Bio-Rad Laboratories, Richmond, CA) was used for extraction of solution fluoride in a ratio of 1 g HAP per liter of solution (when necessary, the solution was pre-adjusted to a determined higher pH so that the HAP would not dissolve). The slurry was agitated for 24 hours and then filtered to obtain the preliminarily de-fluoridated solution. To ensure that extremely low levels of fluoride residue remained in the solution,
low fluoride-containing CAP, synthesized in this laboratory, was utilized to further extract fluoride from the preliminarily de-fluoridated solution in a ratio of 100 mg of CAP per liter of solution. The slurry was agitated for 48 hours and filtered to obtain the final de-fluoridated solution.

**Low Fluoride-containing CAP Synthesized by Hydrolysis Method**

This synthesis method follows the procedure initially reported by LeGeros *et al.* (14) with some modification. In order to obtain low fluoride-containing CAP, all stock solutions in the following syntheses were defluoridated with the procedure described above. Two hundred forty ml NaH$_2$PO$_4$/Na$_2$HPO$_4$ stock solution (0.42 M / 0.42 M) and 240 ml CaCl$_2$ (0.83 M) were slowly added (1 ml/min) into 3.5 liters of doubly deionized water at room temperature. After the precipitate mixture was digested for 1 hour, it was filtered and then dried at 60°C overnight. The product was determined to be dicalcium phosphate dihydrate (DCPD, CaHPO$_4$·2H$_2$O) by X-ray diffraction and chemical analysis. DCPD was then converted to dicalcium phosphate (DCP, CaHPO$_4$) by heating at 100°C for 1 hour. Ten g of DCP were suspended in 4 liters of doubly deionized water containing a predetermined level of NaHCO$_3$. The mixture was then brought to predefined temperature and maintained for 48 hours with stirring for hydrolysis. The residue was obtained by filtering the mixture and washing three times with doubly deionized water. The final residue was then dried at 60°C for 24 hours. A 6% CAP preparation was synthesized for this study and it contained about 1 ppm of fluoride.
Low Fluoride-containing CAP Synthesized by Precipitation Method

This method followed the precipitation method as described by Nelson and Featherstone (15) with some modification. Again, as described above, all stock solutions were defluoridated prior to use. Calcium nitrate solution (200 ml; 0.3 M) and sodium dihydrogen phosphate solution (200 ml; 0.18 M) with different amount of sodium bicarbonate were added dropwisely with a peristaltic pump at a rate of 1.4 ml/min into 3 L of doubly deionized water with temperature maintained at 70, 85 or 95 °C. The pH of the mixture was kept constant at 9.0±0.1 with NaOH solution (1 M). After the addition of the calcium and the phosphate/carbonate solutions was completed, the mixture was digested for an additional hour. The synthesized CAP was obtained by filtering the mixture and washing three times with doubly deionized water. The final residue was then dried at 60°C for 24 hours. CAP preparations were synthesized by this method at different carbonate levels (1-7%) at three predefined temperatures for this study, and they contain about 0.6 ppm of fluoride.

MES Determination

The procedures for the determination of MES distributions were based on the method described previously (13) with some changes. A series of 0.1 M acetate buffer solutions was prepared by mixing calculated amounts of AR-grade CaCl₂ (Mallinckrodt) and NaH₂PO₄ (Fisher Scientific) stock solutions. The pH and ionic strength were adjusted to their calculated values by NaOH and NaCl, respectively. These partially saturated solutions corresponded to various levels of solution ion activity product $I_{HAP}$. The calculations for the driving force function $I_{HAP}$ were done with the computer program
CHEMIST (MicroMath Scientific Software, Salt Lake City, UT). These solutions were then defluoridated with the method described previously.

Experiments which used the CAP prepared by the hydrolysis method were done with accurately weighed 40 mg (solutions at pH 4.5) or 10 mg (solutions at pH 6.5) CAP powder suspended in 1 L of each solution. Experiments which used CAPs prepared by the precipitation method were done with accurately weighed 10 mg (solutions at any pH) CAP powder suspended in 2 L of each solution. The system was allowed to equilibrate at 30°C for 48 h under stirring. After equilibration, the suspensions were filtered and rinsed with doubly deionized water. The CAP residue was dissolved with 0.1 M perchloric acid, and was then analyzed for calcium and phosphate with photometric methods (16, 17). With the original sample as the control, the fraction of dissolved CAP was obtained from the amount of undissolved residue and the initial CAP. The percentage of dissolved CAP was then plotted against the solution p$\text{H}_{\text{HAP}}$ to construct the MES distribution plot.

In this study, predetermined amounts of fluoride were added into partially saturated solutions after the solutions were defluoridated and before the MES equilibration was started. After each MES was determined, the concentration of the solution fluoride was analyzed using the procedure described in the following paragraphs.

**Determination of Solution Fluoride in Sub-ppb Levels**

Three main steps were involved in this technique. First, CAP powder was used as an adsorbent to quantitatively extract solution fluoride. Then the adsorbed fluoride was separated from the CAP powder and transferred into a solution suitable for the analysis
using a fluoride-selective electrode. Finally, the fluoride concentration was determined by the fluoride-selective electrode. These steps are itemized in the next three paragraphs.

A predetermined amount of low fluoride-containing CAP powder (e.g., 100 mg) was suspended in the test solution and allowed to equilibrate at room temperature under stirring. Sometimes, the pH of test solutions needed to be adjusted to a higher value prior to the equilibration in order that the CAP absorbent was not dissolved. After a certain equilibration time (see Results and Discussion for details), the mixture was filtered and rinsed with doubly deionized water. The recovered CAP powder was dried in an oven at 60°C for 24 hours. Then the dried CAP powder was weighed and transferred to a diffusion cell.

The fluoride diffusion technique followed the Taves (18) method with some modification. The diffusion cell was made by placing one plastic Petri dish inside of another larger one and attaching them with glue. In the center chamber, 1.0 ml of 0.2 M NaOH was added. Either CAP with adsorbed fluoride or a fluoride standard, 5 ml of 3 M HClO₄, and 0.5 ml of hexamethyldisiloxane (HMDS, [(CH₃)₃Si]₂O) saturated 5 M HCl were added in different sectors of the outer chamber. The cell was sealed by parafilm and then rotated for 1 min to mix all reagents of the outer compartment. After 4 hours of diffusion at room temperature, 0.9 ml NaOH solution in the center chamber was taken and mixed with 0.1 ml of 2 M acetic acid. The final sample size was 1 ml at pH 5.5, which was appropriate for the fluoride analysis using a fluoride-selective electrode.

Fluoride electrodes from two manufacturers were used in this study: Radiometer America Inc. (type FK1502F) and Corning Inc. (Catalog No. 34108-490). In order to obtain optimal analytical results, these electrodes were polished prior to use with
toothpaste and a cloth. The fluoride sample was measured by the electrode under stirring and the equilibration time was 15 min.

**Determination of Threshold Concentrations of Solution**

**Fluoride Affecting Solubility of CAPs**

This experiment was composed of two procedures described previously: determination of MES, and analysis of solution fluoride concentration. A certain amount of CAP sample was equilibrated in a partially saturated solution with predetermined solution $I_{\text{HAP}}$, pH, as well as known amount of added fluoride (including zero added fluoride). After equilibration for 48 h, the undissolved CAP residue was analyzed and the percent of dissolved apatite in this solution could be obtained (see MES Determination). In the meantime, the filtrate was collected and the free solution fluoride concentration could be measured using the procedure described previously.

In order to examine the inhibitory effect of solution fluoride on the solubility (MES) of a CAP sample, the CAP MESs were determined in a series of solutions with the same solution $I_{\text{HAP}}$ and pH but with various amounts of initially added fluoride. The lowest concentrations of solution fluoride (the threshold) influencing the solubility behavior of the CAP could be investigated when the CAP MES was plotted as a function of free solution fluoride concentration (i.e., the concentration of solution fluoride at the end of the solubility studies).
Results and Discussion

Proper Selection of Fluoride-selective Electrodes of High Sensitivity and Reproducibility

The low limit of most fluoride-selective electrodes was claimed as 0.02 ppm by the manufacturers. However, in order to obtain reliable results for samples with fluoride less than 1 ppm, many precautions needed to be kept in mind. For example, the fluoride levels in Total Ionic Strength Adjustment Buffer (TISAB) solutions are often high enough to compromise the results. In this study, fluoride selective electrodes from several manufacturers were tested, and two models were found to be capable of directly measuring solution fluoride as low as 0.02 ppm accurately when the sample size was 1 ml.

Figure 6.1 shows the response curve of a F-selective electrode which was manufactured by Radiometer America Inc. (type FK1502F). Whereas the response curve follows the Nernst Equation when the solution fluoride concentration is higher than 1 ppm, the responses deviate from the Nernst Equation for fluoride concentrations in the range of 0.02 ppm to 1 ppm. As a consequence, a second-order polynomial equation was used to fit the results, and served as the standard curve for fluoride concentrations in the range of 0.02 – 50 ppm. Table 6.1 shows the results obtained with this electrode at low fluoride concentrations, i.e., less than 0.1 ppm (all test electrodes were found to yield good results at high fluoride levels). This electrode provided good sensitivity and reproducibility. Solution fluoride concentrations down to 0.02 ppm for sample sizes as small as 1 ml could be conveniently measured. Comparable results were obtained with a fluoride electrode from Corning Inc. (Catalog No. 34108-490). These two electrodes were found to be the best among others investigated. It is important to note that prior to
Figure 6.1 Standard curve of the fluoride-selective electrode in the range from 0.02 ppm to 50 ppm. A second order polynomial curve is used to fit the data.
Table 6.1 Radiometer fluoride electrode (FK1502K) tested in 1ml TISAB.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Prepared test solution (ppm)</th>
<th>Results from electrode reading (ppm)</th>
<th>Relative error (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.022</td>
<td>0.022</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.025</td>
<td>0.024</td>
<td>-4.0</td>
</tr>
<tr>
<td>3</td>
<td>0.030</td>
<td>0.032</td>
<td>6.7</td>
</tr>
<tr>
<td>4</td>
<td>0.044</td>
<td>0.045</td>
<td>2.3</td>
</tr>
<tr>
<td>5</td>
<td>0.074</td>
<td>0.071</td>
<td>-4.1</td>
</tr>
</tbody>
</table>
each fluoride-determination experiment, the F-selective electrode was polished and then equilibrated in doubly deionized water for a time period of about 20 min.

**Modified Taves Diffusion Method**

The diffusion method for fluoride measurement was vastly improved by Taves (18). The use of HMDS makes it possible to separate fluoride from samples by diffusion at room temperature in a short time (e.g., several tens of minutes). Taves believed that HMDS acts as a catalyst to accelerate the diffusion of fluoride. He suggested that during diffusion the HClO$_4$ solution in the outer chamber convert free F$^-$ ions into molecular HF. Without HMDS in the solution, HF tends to form multiple complexes which are much less volatile than HF itself. With HMDS, however, HF and HMDS tend to form a HF-HMDS complex which is highly volatile. When the HF-HMDS complex contacts the NaOH solution in the center chamber, HF is released, and HMDS can diffuse back to the outer chamber.

Taves found that if the diffusion cell was kept in rotary motion, fluoride could be recovered for more than 98% within 1 hour, while 3 hours would be enough to complete the diffusion if the diffusion cell was held stationary. In our experiments, the recovery of fluoride was also tested. It was found that the recovery is more than 95% if diffusion takes place in a stationary diffusion cell for more than 3 hours (see Table 6.2).

It was also reported (18) that stock HCl is contaminated with appreciable amounts of fluoride. In our study, fluoride recoveries were found to be significantly higher than 100% for samples with fluoride less than 0.1 ppm. Further tests indicated that not only HCl but also HClO$_4$ is contaminated with small amounts of fluoride. Efforts were made in
Table 6.2 The recovery of fluoride in the diffusion apparatus.

<table>
<thead>
<tr>
<th>Prepared test solution F (ppm) in outer chamber</th>
<th>Results from diffusion test (ppm) in center chamber</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020</td>
<td>0.026</td>
<td>129.6</td>
</tr>
<tr>
<td>0.050</td>
<td>0.054</td>
<td>108.2</td>
</tr>
<tr>
<td>0.100</td>
<td>0.102</td>
<td>102.1</td>
</tr>
<tr>
<td>0.200</td>
<td>0.204</td>
<td>101.9</td>
</tr>
<tr>
<td>0.500</td>
<td>0.498</td>
<td>99.5</td>
</tr>
<tr>
<td>1.000</td>
<td>1.019</td>
<td>101.9</td>
</tr>
<tr>
<td>3.000</td>
<td>3.010</td>
<td>100.3</td>
</tr>
<tr>
<td>10.00</td>
<td>10.08</td>
<td>100.8</td>
</tr>
<tr>
<td>50.00</td>
<td>48.79</td>
<td>97.6</td>
</tr>
</tbody>
</table>
this study to clean the fluoride contamination in these reagents. Five ml of HMDS were added into 200 ml of 5 M HCl solution or 200 ml of 3 M HClO₄ solution in an arm-equipped Erlenmeyer flask. The flask was connected to a 6-ml vial containing 2 ml of 0.2 M NaOH solution through a plastic tube, and the system was sealed with parafilm. Both solutions in the flask and the vial were under agitation. The fluoride in the HCl or HClO₄ solution was expected to diffuse into the NaOH solution in the vial. The cleaning process lasted for three days, and the NaOH solution in the vial was changed every day. Significant amounts of fluoride were found in the NaOH solution in the vial.

Although all the solutions involved (5 M HCl, 3 M HClO₄, and 0.2 M NaOH) in the diffusion procedure were defluoridated (NaOH solution was defluoridated by CAP powder as described before), the fluoride recovery of the sample of 0.02 ppm fluoride was always higher than 100%, typically about 30% more (see Table 6.2). Consequently, in order to obtain reliable results for each fluoride analysis, diffusion of a series of known fluoride samples was required to construct a standard curve for the determination of fluoride in unknown samples.

Optimal Conditions for Fluoride Extraction from Solutions

Samples with solution fluoride in ppb or sub-ppb levels can not be directly measured by a F-selective electrode. In order to measure solution fluoride at such low concentrations, carbonated apatite having very low fluoride levels (< 0.6 ppm) was used as the adsorbent to quantitatively extract the fluoride out of the test solutions. This step, in the context of the present method, could effectively concentrate the fluoride concentration by more than 1000-fold so that solution fluoride analysis at sub-ppb levels
was feasible using the F-selective electrode. The success of this approach depended upon the following variables: the amount of the carbonated apatite adsorbent used in the extraction, the equilibration time for quantitative extraction, and the volume of the test solutions.

The effect of the amount of CAP used as adsorbent on the solution fluoride recovery efficiency is shown in Table 6.3. The extraction of fluoride from partially saturated solution (Table 6.3a) and doubly deionized water (Table 6.3b) was conducted with different amounts of adsorbent CAP, and the sample fluoride concentrations were prepared to be 10 and 30 ppb for partially saturated solutions and 0.02, 0.05 and 0.10 ppb for doubly deionized water. From these two tests, it could be generalized that greater amounts of adsorbent always provided increase in the percent recovery (see Table 6.3a and b) until all the fluoride in the solution was recovered (see Table 6.3a). Increasing the amount of adsorbent increases the surface area of CAP available for the adsorption of fluoride ions in solution. As a result, the greater the amount of CAP adsorbent used, the higher the recovery percentage was, and eventually a recovery of about 100% was reached. However, since there exists fluoride impurity even in the adsorbent CAP, increasing the amount of CAP also increases the level of background fluoride and thus introduces more fluoride analysis error. From this point of view, the smaller the amount of CAP, the higher the accuracy of this fluoride-determination technique. Therefore, a judicious selection of the amount of CAP used for the fluoride extraction requires optimizing both an increase in fluoride recovery efficiency and a decrease in background fluoride noise.
Table 6.3 Effect of amount of adsorbent CAP on the recovery efficiency.

(a) The test solutions were partially saturated solutions at pH 6.5 and pI_HAP 116.2. The equilibration time was 3 hours and each solution volume was 1 L.

<table>
<thead>
<tr>
<th>Amount of CAP (mg)</th>
<th>Prepared test solution (ppb)</th>
<th>Results from present technique (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0</td>
<td>10.0</td>
<td>7.2</td>
<td>71.7</td>
</tr>
<tr>
<td>300.0</td>
<td>10.0</td>
<td>10.3</td>
<td>103.0</td>
</tr>
<tr>
<td>500.0</td>
<td>10.0</td>
<td>10.2</td>
<td>102.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount of CAP (mg)</th>
<th>Prepared test solution (ppb)</th>
<th>Results from present technique (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0</td>
<td>30.0</td>
<td>12.5</td>
<td>41.7</td>
</tr>
<tr>
<td>300.0</td>
<td>30.0</td>
<td>25.7</td>
<td>85.7</td>
</tr>
<tr>
<td>500.0</td>
<td>30.0</td>
<td>27.5</td>
<td>91.7</td>
</tr>
</tbody>
</table>

(b) The test solution was doubly deionized water. The equilibration time was 2 days and water volume for each test was 1 L.

<table>
<thead>
<tr>
<th>Amount of CAP (mg)</th>
<th>Prepared test solution (ppb)</th>
<th>Results from present technique (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>0.020</td>
<td>0.003</td>
<td>12.7</td>
</tr>
<tr>
<td>40.0</td>
<td>0.020</td>
<td>0.012</td>
<td>62.6</td>
</tr>
<tr>
<td>20.0</td>
<td>0.050</td>
<td>0.020</td>
<td>39.9</td>
</tr>
<tr>
<td>40.0</td>
<td>0.050</td>
<td>0.056</td>
<td>112.2</td>
</tr>
<tr>
<td>20.0</td>
<td>0.100</td>
<td>0.042</td>
<td>42.1</td>
</tr>
<tr>
<td>40.0</td>
<td>0.100</td>
<td>0.070</td>
<td>70.2</td>
</tr>
<tr>
<td>20.0</td>
<td>0.500</td>
<td>0.193</td>
<td>38.6</td>
</tr>
<tr>
<td>40.0</td>
<td>0.500</td>
<td>0.323</td>
<td>64.6</td>
</tr>
</tbody>
</table>
Table 6.3 Contd.

(c) The results of Table 6.3 (a) when the control (background) experimental data were shown.

<table>
<thead>
<tr>
<th>Amount of CAP (mg)</th>
<th>Prepared test solution (added F, ppb)</th>
<th>Results from present technique (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0</td>
<td>0.0</td>
<td>0.07</td>
</tr>
<tr>
<td>100.0</td>
<td>10.0</td>
<td>7.24</td>
</tr>
<tr>
<td>100.0</td>
<td>30.0</td>
<td>12.58</td>
</tr>
<tr>
<td>300.0</td>
<td>0.0</td>
<td>0.11</td>
</tr>
<tr>
<td>300.0</td>
<td>10.0</td>
<td>10.41</td>
</tr>
<tr>
<td>300.0</td>
<td>30.0</td>
<td>25.82</td>
</tr>
<tr>
<td>500.0</td>
<td>0.0</td>
<td>0.17</td>
</tr>
<tr>
<td>500.0</td>
<td>10.0</td>
<td>10.37</td>
</tr>
<tr>
<td>500.0</td>
<td>30.0</td>
<td>27.68</td>
</tr>
</tbody>
</table>

(d) The results of Table 6.3 (b) when the control (background) experimental data were shown.

<table>
<thead>
<tr>
<th>Amount of CAP (mg)</th>
<th>Prepared test solution (added F, ppb)</th>
<th>Results from present technique (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>0.00</td>
<td>0.020</td>
</tr>
<tr>
<td>20.0</td>
<td>0.02</td>
<td>0.023</td>
</tr>
<tr>
<td>20.0</td>
<td>0.05</td>
<td>0.040</td>
</tr>
<tr>
<td>20.0</td>
<td>0.10</td>
<td>0.062</td>
</tr>
<tr>
<td>20.0</td>
<td>0.50</td>
<td>0.213</td>
</tr>
<tr>
<td>40.0</td>
<td>0.00</td>
<td>0.019</td>
</tr>
<tr>
<td>40.0</td>
<td>0.02</td>
<td>0.031</td>
</tr>
<tr>
<td>40.0</td>
<td>0.05</td>
<td>0.075</td>
</tr>
<tr>
<td>40.0</td>
<td>0.10</td>
<td>0.089</td>
</tr>
<tr>
<td>40.0</td>
<td>0.50</td>
<td>0.342</td>
</tr>
</tbody>
</table>

1 The amount of adsorbent (CAP) used for the extraction of solution fluoride.
2 The concentration of fluoride initially prepared in solution.
3 The concentration of fluoride initially prepared in water.
4 The concentration of fluoride determined by the present procedure. The background solution fluoride concentrations were deducted.
5 The concentration of fluoride determined by the present procedure. The background solution fluoride concentrations were included.
The effect of equilibration time on the extraction of solution fluoride was investigated with solutions at pH 4.5 and $p_{\text{HAP}}$ 113. The solutions were spiked with fluoride to predetermined levels and then the fluoride concentrations of the solutions were measured by the present method at different equilibration times. Results (see Table 6.4) showed that longer equilibration times (i.e., 20 days) increase the average percent of fluoride recovery in both cases considered herein, i.e., solution fluoride concentrations of 0.05 ppb and 0.10 ppb. While greater equilibration times provided some increase in the recovery, this made the experiment very inconvenient and it was felt unjustified to use longer equilibration times (e.g., 10 or 20 days) in view of the total variability. To make the fluoride extraction procedure feasible in practical experiments, efforts were made to reduce the equilibration time to a few days with a satisfactory percent of solution fluoride recovery.

When the volume of unknown solution was 1000 ml, in the context of the present fluoride analysis method, the solution fluoride concentration could be concentrated by 1000 times since the final sample size which was directly quantified using the F-selective electrode was 1.0 ml. In order to increase the sensitivity of this method, a solution volume greater than 1.0 liter (i.e., 2.0 liters) was tested with the expectation that the solution fluoride recovery rate would be improved when the equilibration time was not too long (i.e., a few days). As can be seen from the experimental results given in Table 6.5, there was a significant gain in going from 1.0 liter solution volume to 2.0 liter volume for fluoride extraction when the equilibration time was 5 days. Therefore, increasing the solution volume is an effective way to improve the sensitivity and accuracy of the present fluoride analysis procedure.
Table 6.4 Effect of equilibration time on the solution fluoride recovery efficiency. (a) Test samples were partially saturated solutions at pH 4.5 and $pI_{HAP}$ 113. The amount of CAP used for fluoride extraction was 40 mg and each solution volume was 1 L.

<table>
<thead>
<tr>
<th>Equilibration time (day)</th>
<th>Prepared test solution (ppb)</th>
<th>Results from present technique (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.050</td>
<td>0.017</td>
<td>34.7</td>
</tr>
<tr>
<td>5</td>
<td>0.050</td>
<td>0.024</td>
<td>47.6</td>
</tr>
<tr>
<td>10</td>
<td>0.050</td>
<td>0.022</td>
<td>44.0</td>
</tr>
<tr>
<td>20</td>
<td>0.050</td>
<td>0.039</td>
<td>78.7</td>
</tr>
<tr>
<td>2</td>
<td>0.100</td>
<td>0.041</td>
<td>41.1</td>
</tr>
<tr>
<td>5</td>
<td>0.100</td>
<td>0.046</td>
<td>45.5</td>
</tr>
<tr>
<td>10</td>
<td>0.100</td>
<td>0.088</td>
<td>88.2</td>
</tr>
<tr>
<td>20</td>
<td>0.100</td>
<td>0.101</td>
<td>101.1</td>
</tr>
</tbody>
</table>

(b) The results of Table 6.4 (a) when the control (background) solution fluoride concentrations were included.

<table>
<thead>
<tr>
<th>Equilibration time (day)</th>
<th>Prepared test solution (added F, ppb)</th>
<th>Results from present technique (ppb)</th>
<th>Apparent recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.00</td>
<td>0.032</td>
<td>98.0</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.049</td>
<td>98.0</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.073</td>
<td>73.0</td>
</tr>
<tr>
<td>5</td>
<td>0.00</td>
<td>0.033</td>
<td>114.0</td>
</tr>
<tr>
<td>5</td>
<td>0.05</td>
<td>0.057</td>
<td>114.0</td>
</tr>
<tr>
<td>5</td>
<td>0.10</td>
<td>0.079</td>
<td>79.0</td>
</tr>
<tr>
<td>10</td>
<td>0.00</td>
<td>0.035</td>
<td>114.0</td>
</tr>
<tr>
<td>10</td>
<td>0.05</td>
<td>0.057</td>
<td>114.0</td>
</tr>
<tr>
<td>10</td>
<td>0.10</td>
<td>0.123</td>
<td>123.0</td>
</tr>
<tr>
<td>20</td>
<td>0.00</td>
<td>0.029</td>
<td>136.0</td>
</tr>
<tr>
<td>20</td>
<td>0.05</td>
<td>0.068</td>
<td>136.0</td>
</tr>
<tr>
<td>20</td>
<td>0.10</td>
<td>0.130</td>
<td>130.0</td>
</tr>
</tbody>
</table>

1 The equilibration time used for the extraction of solution fluoride by CAP.
2 The concentration of fluoride initially prepared in solution.
3 The concentration of fluoride determined by the present procedure. The background solution fluoride concentrations were deducted.
4 The concentration of fluoride determined by the present procedure. The background solution fluoride concentrations were included.
Table 6.5 Effect of test solution volume on the solution fluoride recovery efficiency. (a) Test samples were partially saturated solutions at pH 4.5 and pHAP 113. The amount of CAP used for fluoride extraction was 40 mg, and the equilibration time was 5 days.

<table>
<thead>
<tr>
<th>Test solution volume (L)</th>
<th>Prepared test solution (ppb)</th>
<th>Results from present technique (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.020</td>
<td>0.014</td>
<td>70.7</td>
</tr>
<tr>
<td>2.0</td>
<td>0.020</td>
<td>0.027</td>
<td>135.4</td>
</tr>
<tr>
<td>1.0</td>
<td>0.050</td>
<td>0.022</td>
<td>44.0</td>
</tr>
<tr>
<td>2.0</td>
<td>0.050</td>
<td>0.042</td>
<td>84.8</td>
</tr>
<tr>
<td>1.0</td>
<td>0.100</td>
<td>0.054</td>
<td>54.4</td>
</tr>
<tr>
<td>2.0</td>
<td>0.100</td>
<td>0.086</td>
<td>86.0</td>
</tr>
</tbody>
</table>

(b) The results of Table 6.5 (a) when the control (background) solution fluoride concentrations were included.

<table>
<thead>
<tr>
<th>Test solution volume (L)</th>
<th>Prepared test solution (added F, ppb)</th>
<th>Results from present technique (ppb)</th>
<th>Apparent recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.00</td>
<td>0.035</td>
<td>245.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.02</td>
<td>0.049</td>
<td>114.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.05</td>
<td>0.057</td>
<td>89.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.10</td>
<td>0.089</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>0.00</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>0.02</td>
<td>0.067</td>
<td>335.0</td>
</tr>
<tr>
<td>2.0</td>
<td>0.05</td>
<td>0.062</td>
<td>124.0</td>
</tr>
<tr>
<td>2.0</td>
<td>0.10</td>
<td>0.126</td>
<td>126.0</td>
</tr>
</tbody>
</table>

1 The test solution volume from which fluoride would be extracted.
2 The concentration of fluoride initially prepared in solution.
3 The concentration of fluoride determined by the present procedure. The background solution fluoride concentration (0.04 ppb) was deducted.
4 The concentration of fluoride determined by the present procedure. The background solution fluoride concentrations were included.
Since the developed fluoride-determination procedure is laborious and sensitive to variables and the solution fluoride concentration is concentrated by more than 1000-fold, much care is required to conduct this experiment to avoid potential experiment errors. For example, the use of a glass stirring bar, instead of a Teflon stirring bar, is suggested to agitate the slurry during the fluoride-extraction process since it was found that trace amounts of fluoride in the Teflon stirring bar could contaminate the test solution, even though the fluoride was supposed to be chemically bound to carbon in the polymer. Furthermore, because it has been reported that fluoride can be adsorbed on the wall of glassware, fluoride concentrations at sub-ppb levels were measured using both glass beakers and plastic beakers as containers. The results (not shown here) did not demonstrate any significant difference between the two types of beakers. Lastly, since the pH of the partially saturated solutions needed to be adjusted prior to fluoride extraction, in order to minimize the introduction of unwanted fluoride into the solutions, stock solutions of NaOH and HCl, which were used to adjust the pHs of partially saturated solutions, were defluoridated to remove the fluoride impurity by the methods described earlier.

According to the previous analysis and many independent validation tests using the present fluoride determination procedure, the following conditions are suggested for fluoride extraction and quantification of unknown solutions. When the solution fluoride concentrations were below 0.1 ppb, 40 mg of CAP adsorbent is used to extract the unknown solution fluoride in a volume of 2 liters and the equilibration time is 5 days. At these fluoride levels, the lowest sensitivity of this technique is about 0.02 ppb. While the fluoride assay error was around 10% when fluoride concentration was 0.1 ppb, the experimental error increased to ~20% when solution fluoride level was 0.05 ppb, and the
error increased to 30 - 40% when solution fluoride concentration is 0.02 ppb. The average experiment error was around 20% and, obviously, the lower the fluoride concentrations the greater the fluoride assay error. When the solution fluoride levels were in the range of 0.1 to 10.0 ppb, 100 mg of CAP was equilibrated with 2 liters of unknown solution for 2 days for the extraction of solution fluoride. The experiment error was estimated to be 10% in these fluoride concentrations. If the solution fluoride concentration is about 10 to 30 ppb, the solution volume and equilibration time was maintained at 2 liters and 2 days, respectively, and the amount of CAP adsorbent was increased to 300 mg. When a solution contains even higher levels of fluoride (> 30 ppb), a F-selective electrode can be directly applied to measure the solution fluoride concentration and the solution fluoride extraction process becomes unnecessary.

**Evaluation of the Defluoridation Procedure**

Using commercial HAP to extract fluoride impurity from dissolution media has been reported before (19, 20). To verify the outcome of this defluoridation procedure, two successive fluoride extractions were employed to remove the fluoride from the partially saturated solutions. In each extraction, 1 g of commercial Bio-Gel HTP HAP was used for 1 L solution. The fluoride levels in the solutions after defluoridation could decrease to as low as about 0.10 ppb. Since the commercial HAP contains about 20 ppm fluoride, low fluoride-containing CAP (< 1 ppm) was tried as the adsorbent. Using low fluoride-containing CAP as the fluoride extraction adsorbent, I found that solutions fluoride concentrations could reach as low as about 0.04 ppb. Other calcium phosphates
may also be good choices as fluoride extraction adsorbents, but it requires that they contain low fluoride impurity and they are of low solubility.

In this study, efforts were made to explore the lowest solution (including doubly deionized water) fluoride level that could be reached using this defluoridation procedure. Since it was known that the fluoride in solution was extracted by the present procedure, the procedure was repeatedly employed to analyze the solution fluoride concentration with an effort to estimate the lowest fluoride levels obtainable. Such results for doubly deionized water are shown in Table 6.6a. For some unknown reason, the fluoride concentration of doubly deionized water was always around 0.02 ppb no matter how many times the water was defluoridated by the present method. This fluoride concentration could be considered either as the lowest solution fluoride level the defluoridation procedure can get or as the noise signal level of this fluoride analysis method. Similar phenomena were also found with partially saturated solutions (see Table 6.6b). A lower fluoride concentration (around 0.05 ppb) could always be measured in partially saturated solutions even if the solutions were “cleaned” repeatedly by the present fluoride extraction procedure.

It is worth noticing that using either commercial HAP or synthesized CAP for fluoride extraction somehow affect the solution concentrations of calcium and phosphate. HAP or CAP was found to release a small amount of calcium and phosphate into the solution, and/or adsorb some calcium and phosphate ions from the solution. Therefore, in order to control the solution ion activity product after defluoridation, solution concentrations of calcium and phosphate were measured experimentally.
Table 6.6 Solution fluoride concentrations after multiple CAP extractions.

(a) Doubly deionized water with and without added fluoride was tested. 40 mg CAP adsorbent and a volume of 1 L deionized water were equilibrated for 2 days for each fluoride extraction.

<table>
<thead>
<tr>
<th>Fluoride added (ppb)</th>
<th>1st extraction (ppb)</th>
<th>2nd extraction (ppb)</th>
<th>3rd extraction (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.013 ± 0.003 *</td>
<td>0.021 ± 0.007 *</td>
<td>0.016 ± 0.004 *</td>
</tr>
<tr>
<td>0.10</td>
<td>0.078</td>
<td>0.043</td>
<td>0.027</td>
</tr>
</tbody>
</table>

* The standard deviation of three independent measurements.

(b) Partially saturated solutions with and without added fluoride were tested. 40 mg CAP adsorbent and a volume of 2 L solution (pH 4.5 and pI\textsubscript{HAP} 113.5) were equilibrated for 5 days for each fluoride extraction.

<table>
<thead>
<tr>
<th>Fluoride added (ppb)</th>
<th>1st extraction (ppb)</th>
<th>2nd extraction (ppb)</th>
<th>3rd extraction (ppb)</th>
<th>4th extraction (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>0.113</td>
<td>0.049</td>
<td>0.050</td>
<td>0.054</td>
</tr>
<tr>
<td>0.070</td>
<td>0.180</td>
<td>0.087</td>
<td>0.058</td>
<td>0.054</td>
</tr>
</tbody>
</table>
Preliminary Study of Solution Fluoride Threshold Concentrations for a CAP Sample Synthesized by the Hydrolysis Method

The CAP sample used in this study, synthesized by the hydrolysis method at 95°C, is denoted as CAP H956 (H represents hydrolysis). CAP H956 was determined to possess carbonate content of 6% by weight, and surface area of 13.4 m²/g. The MES of this CAP was quantified in defluoridated buffer solutions at pH 4.5 and pI_HAP ranging from 113 to 123; the MES distribution profile is shown in Figure 6.2. The primary purpose of this study was to investigate the lowest solution fluoride concentration (threshold) at which fluoride began to affect the CAP solubility. Since recent studies in this laboratory have focused on CAP samples synthesized by the precipitation method, studies of threshold solution fluoride levels for this hydrolysis synthesis CAP sample were groundbreaking.

The first investigation in this study was the relationship of the CAP MES and solution free-fluoride level at pH 4.5 and pI_HAP 115.4. The initial solid-to-solution ratio of this experiment was 40 mg CAP per 1000 mL solution. All the experiments were done at the same pI_HAP and pH but with various amounts of initially added fluoride. Since significant amount of solution fluoride was adsorbed on the surface of CAP crystals, the concentrations of solution free-fluoride were measured after the equilibration period, at the same time that the CAP MES was determined. Figure 6.3 shows the solution free-fluoride measurements after the equilibration and the corresponding amount of initially added fluoride. As can be seen, up to the concentration of 3 ppb added fluoride, the free fluoride concentrations determined in the end of MES test were indistinguishable from 0. At least two reasons account for this observation: large amounts of CAP H956 (40 mg) were used in equilibration, thus most of the fluoride ions were adsorbed onto the surface
Figure 6.2 MES distribution of CAP H956 (6% carbonate). The MES determination experiment was performed in defluoridated solutions. The best fit curve is based on equation $y = 50\{1 + \text{erf}[k(x-x_0)]\}$, where $\text{erf}$ is the error function.
Figure 6.3 Measured concentrations of free solution fluoride in the end of CAP H956 MES determination experiments at pH 4.5 and pH_{HAP} 115.4. The broken line indicates the concentration of fluoride in the region of 0 ppb.
of the CAP residue; and the fluoride-analysis technique employed at the moment was not sensitive to detect such low levels of solution fluoride. Therefore, when 10 ppb of fluoride was initially doped into the solution, there was only 0.04 ppb left as free fluoride in the solution after equilibration; the measurement of 0.04 ppb was estimated to have an experimental error of 50%. Figure 6.4 presents the CAP percentage dissolved (MES) versus the concentrations of free solution fluoride at the end of a two-day equilibration. The results show that the threshold concentration of free solution fluoride affecting the solubility behavior of the CAP preparation is extremely low in solutions of pH 4.5 and pI_{HAP} 115.4, which was around 0.04 ppb (or $2 \times 10^{-9}$ M).

The same investigations were also conducted in solutions at pH 6.5 and two pI_{HAP} conditions: 115.2 and 116.2. Let us first examine the solution free-fluoride concentrations after the MES determination test and the corresponding added fluoride concentrations, which are demonstrated in Figure 6.5. As can be seen, most of solution fluoride was adsorbed on the CAP when 0.3 or 0.6 ppb fluoride was added. As a comparison, a small percent of solution fluoride was further adsorbed on the CAP when the added fluoride was 3 or 5 ppb, and this indicated that it was near the saturation when the added fluoride was 3 or 5 ppb. This figure has also shown that the adsorption of solution fluoride on the apatite surface is pH dependent, i.e., the solution free-fluoride concentration was much higher at pH 6.5 than that at pH 4.5 when the same amount of fluoride was initially added into the solution of identical pI_{HAP}. This result was also found by Bergstrom (19). According to his explanation, OH$^-$ and F$^-$ ions may be competitively binding to adsorption sites on the apatite surface. At higher pH, the concentration of OH$^-$ is greater so that the adsorption of F$^-$ is inhibited.
Figure 6.4 Effect of free solution fluoride on solubility of CAP H956 in solutions at pH 4.5 and pI/HAP of 115.4.
Figure 6.5 Measured concentrations of free solution fluoride in the end of CAP H956 MES determination experiments at pH6.5. The experiments were performed in solutions of $p\text{I}_{\text{HAP}}$ 115.2 ($\blacksquare$) and in solutions of $p\text{I}_{\text{HAP}}$ 116.2 (O).
Figures 6.6 and 6.7 show the effect of low solution fluoride (at pH 6.5) on the solubility (MES) of CAP H956 in solutions of pH\textsubscript{HAP} 116.2 and 115.2. The results show that the threshold fluoride concentration for this CAP sample was between 0.4 ppb to 0.8 ppb and between 0.1 ppb to 0.2 ppb in solution pH\textsubscript{HAP} of 116.2 and 115.2, respectively. Below these threshold levels, solution fluoride has minor or negligible inhibitory effect on the CAP solubility.

The variation of fluoride threshold level in different solution conditions suggests that the fluoride threshold concentration depends on the solution composition, i.e., solution pH and pH\textsubscript{HAP}. Further analysis of solution pH\textsubscript{FAP} in these conditions also suggested that, for a given CAP sample, fluoride threshold levels could be related to solution pH\textsubscript{FAP}. Solutions at pH 4.5 contain about 100 times fewer OH\textsuperscript{-} ions than solutions at pH 6.5. For two solutions of same solution pH\textsubscript{FAP}, concentrations of calcium and phosphate in the pH 4.5 solution are greater than that in the pH 6.5 solution. Consequently, a much lower concentration of fluoride is required to reach a certain value of pH\textsubscript{FAP} at a lower pH than that at a higher pH. Thus the fluoride threshold concentration for CAP H956 is much less at pH 4.5 than at pH 6.5. Likewise, solutions with a greater pH\textsubscript{HAP} value contain lower concentrations of calcium and phosphate and more fluoride is needed to reach a certain level of solution pH\textsubscript{FAP}. As a result, the fluoride threshold level is lower in more concentrated solutions (e.g., pH\textsubscript{HAP} of 115.2) than that in the less concentrated solutions (e.g., pH\textsubscript{HAP} of 116.2).

Investigations of solution fluoride threshold concentrations for CAP H956 were preliminary and, as can be see, the experimental data were scattered. However, many fundamental properties between fluoride thresholds and solution conditions (such as pH
Figure 6.6 Effect of free solution fluoride on solubility of CAP H956 in solutions at pH 6.5 and $p_{\text{HAP}}$ of 116.2.
Figure 6.7 Effect of free solution fluoride on solubility of CAP H956 in solutions at pH 6.5 and pI\textsubscript{HAP} of 115.2.
and $p_{\text{HAP}}$ were revealed. Definitely, this has provided a valuable foundation for further studies of solubility behavior of CAPs at extremely low solution fluoride concentrations.

**Attempts to Determine Solution Fluoride Threshold at pH 4.5 for a CAP Sample Synthesized by the Precipitation Method**

Since solution fluoride threshold levels were extremely low at pH 4.5 solutions, and previous studies showed that there existed large experimental errors, further attempts were made to measure the lowest concentrations of solution fluoride affecting the solubility of apatites. Because most of the recent studies in this laboratory have focused on CAP samples synthesized by the precipitation method, the CAP sample used in this study was prepared by a precipitation procedure at 95°C, hereafter referred to as CAP P957 hereafter (P denotes precipitation). The sample incorporated 7.5% carbonate and possessed a crystallinity (microstrain parameter) value of 0.39.

The partially saturated calcium phosphate solutions were prepared to have an ion activity product $I_{\text{HAP}}$ of about $10^{-114}$. From preliminary experiments, CAP P957 was expected to dissolve to the extent of 20 to 30% in this solution in the absence of solution fluoride. In order to increase the sensitivity of the fluoride measurement, accurately weighed 10 mg of the CAP sample was equilibrated in 2 liters of the prepared partially saturated solution with or without added fluoride. After 2 days of equilibration with agitation, the CAP residue was collected and the amounts of calcium and phosphate were analyzed to determine the amount of the CAP dissolved. The filtrate was collected after the equilibration period, and the solution fluoride levels were measured by the developed fluoride-determination method.
The results of two sets of duplicated studies are presented in Figures 6.8 and 6.9, and the corresponding data are given in Table 6.7. While there is significant data scatter, there is a marked drop in the percent CAP dissolved in both sets of data at solution fluoride around 0.07 to 0.08 ppb. Accordingly, it is proposed that the threshold solution fluoride is about 0.07 to 0.08 ppb for this CAP sample at solution conditions of pH 4.5 and pI_HAP around 113.8 to 114.0. Such results suggest that solution fluoride concentrations (at pH 4.5) should be reduced less than 0.07 ppb in order to study the solubility behavior of CAP P957 without the interference of solution fluoride ions. As will be discussed in the later section of this chapter, many reagents (e.g., AR grade CaCl₂ and CaCO₃ from Mallinckrodt, Inc., Paris, Kentucky) contain fluoride impurity and solutions made by these reagents possess a fluoride concentration greater than the threshold level. Consequently, although no fluoride was purposely added into these solutions, apatite solubility studies in such solutions were affected by the contaminated fluoride. Therefore, investigations of apatite solubility in the absence of solution fluoride are compromised unless the dissolution media has been defluoridated.

Systematic Study of Solution Fluoride Threshold for CAP

Samples Synthesized by the Precipitation Method

In order to conduct a systematic study of the threshold (i.e., lowest) concentrations of solution fluoride that may cause apatite solubility reduction, three CAP samples over a range of crystallinities were synthesized by the precipitation method at 95, 85, and 70°C with carbonate contents in the range of 1 to 6% by weight. The names of the three CAP samples and their main characteristics related to this study are shown in
Figure 6.8 Threshold concentration of solution fluoride affecting the solubility of CAP P957 at pH 4.5 and $p_{\text{HAP}}$ of 113.8. Symbol (□) represents the duplicated experiments of symbol (O).
Figure 6.9 Threshold concentration of solution fluoride affecting the solubility of CAP P957 at pH 4.5 and pH$_{\text{HAP}}$ of 114.0. Symbol (□) represents the duplicated experiments of symbol (O).
Table 6.7 Results of the fluoride threshold experiments for CAP P957 in partially saturated solutions of pH 4.5.

<table>
<thead>
<tr>
<th>Initially added F (ppb)</th>
<th>Free F measured (ppb)</th>
<th>Free F measured (M)</th>
<th>CAP dissolved (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>pI&lt;sub&gt;HAP&lt;/sub&gt; = 113.8</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.064</td>
<td>3.37E-09</td>
<td>21.7</td>
</tr>
<tr>
<td>0.1</td>
<td>0.025</td>
<td>1.32E-09</td>
<td>21.0</td>
</tr>
<tr>
<td>0.3</td>
<td>0.028</td>
<td>1.47E-09</td>
<td>22.3</td>
</tr>
<tr>
<td>1.0</td>
<td>0.033</td>
<td>1.74E-09</td>
<td>22.6</td>
</tr>
<tr>
<td>3.0</td>
<td>0.073</td>
<td>3.84E-09</td>
<td>19.9</td>
</tr>
<tr>
<td>3.0</td>
<td>0.108</td>
<td>5.68E-09</td>
<td>12.1</td>
</tr>
<tr>
<td><strong>pI&lt;sub&gt;HAP&lt;/sub&gt; = 113.8 (Duplicate)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.035</td>
<td>1.84E-09</td>
<td>25.4</td>
</tr>
<tr>
<td>0.1</td>
<td>0.029</td>
<td>1.53E-09</td>
<td>22.6</td>
</tr>
<tr>
<td>1.0</td>
<td>0.032</td>
<td>1.68E-09</td>
<td>19.6</td>
</tr>
<tr>
<td>3.0</td>
<td>0.208</td>
<td>1.09E-08</td>
<td>12.1</td>
</tr>
<tr>
<td>10.0</td>
<td>0.687</td>
<td>3.62E-08</td>
<td>3.1</td>
</tr>
<tr>
<td><strong>pI&lt;sub&gt;HAP&lt;/sub&gt; = 114.0</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.076</td>
<td>4.00E-09</td>
<td>28.9</td>
</tr>
<tr>
<td>0.3</td>
<td>0.079</td>
<td>4.16E-09</td>
<td>28.5</td>
</tr>
<tr>
<td>1.0</td>
<td>0.083</td>
<td>4.37E-09</td>
<td>20.6</td>
</tr>
<tr>
<td>3.0</td>
<td>0.082</td>
<td>4.32E-09</td>
<td>17.0</td>
</tr>
<tr>
<td>10.0</td>
<td>0.103</td>
<td>5.42E-09</td>
<td>8.5</td>
</tr>
<tr>
<td><strong>pI&lt;sub&gt;HAP&lt;/sub&gt; = 114.0 (Duplicate)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.045</td>
<td>2.37E-09</td>
<td>29.4</td>
</tr>
<tr>
<td>0.1</td>
<td>0.039</td>
<td>2.05E-09</td>
<td>29.0</td>
</tr>
<tr>
<td>0.3</td>
<td>0.041</td>
<td>2.16E-09</td>
<td>25.8</td>
</tr>
<tr>
<td>2.0</td>
<td>0.108</td>
<td>5.68E-09</td>
<td>21.1</td>
</tr>
<tr>
<td>10.0</td>
<td>0.586</td>
<td>3.08E-08</td>
<td>7.9</td>
</tr>
<tr>
<td>20.0</td>
<td>0.362</td>
<td>1.91E-08</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Table 6.8. The present research was to further assess the pattern of apatite solubility influence by solution fluoride at the lowest concentrations where fluoride begins to have a solubility inhibiting effect, and to determine the nature of the entity (i.e., the surface complex) that governs the solubility of carbonated apatite under these conditions.

The procedures to measure the CAP solubility (MES) and determine the solution fluoride after equilibration period have been described previously. First, based on preliminary experiments, the three CAP samples were equilibrated in partially saturated solutions at pH 6.5 with the CAP dissolved to the extent of about 60% for each sample in the absence of solution fluoride. After equilibrating the CAP samples in the dissolution media with or without added solution fluoride, the undissolved CAP residues were analyzed to determine the amounts dissolved, and the solution free-fluoride concentrations were measured. Figure 6.10 shows the general pattern of solution fluoride’s effect on the solubility of the three CAP preparations. As can be seen in Figure 6.10, the influence of solution fluoride upon the CAP solubility was gradual. Especially, when solution fluoride concentrations were reduced to less than about 0.04 ppb, the solution fluoride hardly affected the solubility of the CAPs, and hence the solubility behavior of the CAPs could be investigated here without a measurable interference of solution fluoride impurity. Therefore, in practical solubility-determination experiments, a fluoride level of about 0.04 ppb can be taken as the threshold concentration for the CAP samples under these solution conditions. Table 6.9 presents the details of this fluoride threshold concentration study, such as the solution pH\textsubscript{HAP} conditions, amounts of added fluoride, and amounts of fluoride adsorbed on the CAP residues. Figure 6.11 demonstrates the isotherm of fluoride
Table 6.8 Properties of the three precipitation CAP samples investigated in the systematic study of threshold concentrations of solution fluoride.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Synthesis Temperature (°C)</th>
<th>% Calcium</th>
<th>% Phosphate</th>
<th>% Carbonate</th>
<th>Solubility (Mean pK$_{HAP}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP P954</td>
<td>95</td>
<td>38.7</td>
<td>50.1</td>
<td>5.1</td>
<td>116.9</td>
</tr>
<tr>
<td>CAP P851</td>
<td>85</td>
<td>37.7</td>
<td>52.4</td>
<td>1.7</td>
<td>115.2</td>
</tr>
<tr>
<td>CAP P706</td>
<td>70</td>
<td>35.3</td>
<td>48.5</td>
<td>5.2</td>
<td>112.5</td>
</tr>
</tbody>
</table>
Figure 6.10 Threshold concentration of solution fluoride affecting the solubility of CAP P954 (O), CAP P851 (□) and CAP P706 (○) at pH 6.5. Solution $p_{\text{HAP}}$ conditions were 117.0, 115.5 and 112.7 for CAP P954, CAP P851 and CAP P706, respectively. Broken line shows the range of fluoride concentration where fluoride effect on the solubility of CAPs is negligible.
Table 6.9 Results of the fluoride threshold experiments for CAP P954, P851, and P706 in partially saturated solutions of pH 6.5.

<table>
<thead>
<tr>
<th>Initially added F (ppb)</th>
<th>Free F measured (ppb)</th>
<th>CAP dissolved (%)</th>
<th>F adsorption on CAP (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAP P954, $p_{\text{HAP}} = 117.0$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.066</td>
<td>57.7</td>
<td>N/A</td>
</tr>
<tr>
<td>0.0</td>
<td>0.045</td>
<td>58.9</td>
<td>N/A</td>
</tr>
<tr>
<td>0.1</td>
<td>0.070</td>
<td>53.1</td>
<td>0.013</td>
</tr>
<tr>
<td>0.3</td>
<td>0.129</td>
<td>43.8</td>
<td>0.061</td>
</tr>
<tr>
<td>0.6</td>
<td>0.233</td>
<td>28.8</td>
<td>0.103</td>
</tr>
<tr>
<td>1.0</td>
<td>0.413</td>
<td>16.1</td>
<td>0.140</td>
</tr>
<tr>
<td>4.5</td>
<td>1.353</td>
<td>1.5</td>
<td>0.639</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAP P851, $p_{\text{HAP}} = 115.5$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.036</td>
<td>58.8</td>
<td>N/A</td>
</tr>
<tr>
<td>0.0</td>
<td>0.022</td>
<td>59.7</td>
<td>N/A</td>
</tr>
<tr>
<td>0.1</td>
<td>0.037</td>
<td>58.4</td>
<td>0.030</td>
</tr>
<tr>
<td>0.3</td>
<td>0.056</td>
<td>53.5</td>
<td>0.105</td>
</tr>
<tr>
<td>0.6</td>
<td>0.078</td>
<td>47.3</td>
<td>0.198</td>
</tr>
<tr>
<td>1.0</td>
<td>0.142</td>
<td>42.1</td>
<td>0.296</td>
</tr>
<tr>
<td>4.5</td>
<td>0.885</td>
<td>11.2</td>
<td>0.814</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAP P706, $p_{\text{HAP}} = 112.7$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.036</td>
<td>61.3</td>
<td>N/A</td>
</tr>
<tr>
<td>0.0</td>
<td>0.028</td>
<td>60.9</td>
<td>N/A</td>
</tr>
<tr>
<td>0.0</td>
<td>0.029</td>
<td>61.8</td>
<td>N/A</td>
</tr>
<tr>
<td>0.1</td>
<td>0.019</td>
<td>58.9</td>
<td>0.039</td>
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<tr>
<td>0.3</td>
<td>0.036</td>
<td>57.9</td>
<td>0.125</td>
</tr>
<tr>
<td>1.0</td>
<td>0.055</td>
<td>47.6</td>
<td>0.361</td>
</tr>
<tr>
<td>1.0</td>
<td>0.045</td>
<td>46.3</td>
<td>0.356</td>
</tr>
<tr>
<td>3.4</td>
<td>0.124</td>
<td>35.7</td>
<td>1.019</td>
</tr>
<tr>
<td>13.5</td>
<td>0.246</td>
<td>8.9</td>
<td>2.910</td>
</tr>
</tbody>
</table>
Figure 6.11 Fluoride adsorption isotherm for CAP P954 (○), CAP P851 (□) and CAP P706 (◇) in partially saturated solutions of pH 6.5. Slopes of the fitting curves near zero fluoride concentrations are 0.60 (P954), 2.34 (P851) and 11.29 (P706) mg/g/ppb.
adsorption on the apatite residues after the equilibration of the three CAP samples in the dissolution media.

The patterns of solution fluoride affecting the solubility of CAPs at different pHs were investigated in this study. Figure 6.12 presents the inhibitory effect of solution fluoride on CAP P954 at pH 6.5 and pH 5.0. It is clear that the influence of fluoride is more dramatic in solutions of a lower pH value than that of a higher pH value. Since the solution ion activity products for solutions at the two pHs were similar (i.e., $pI_{HAP}$ 117.0 at pH 6.5 and $pI_{HAP}$ 116.7 at pH 5.0), concentrations of calcium and phosphate were greater in the pH 5.0 solutions than that in the pH 6.5 solutions. Bergstrom (19) has reported that hydroxide ions and fluoride ions may competitively bind to adsorption sites on apatite surfaces. Accordingly, hydroxide and fluoride ions may also compete to form the surface complex entity which controls the solubility of the CAP. As will be discussed in the following paragraphs, the functional form governing the CAP solubility becomes more dependent on fluoride as solution fluoride concentrations are increased. Therefore, since the hydroxide ion concentration is about 30 times greater in pH 6.5 solution than that in pH 5.0 solution, fluoride is more potent to affect the apatite solubility in solutions of lower pH when the other solution variables (such as solution $pI_{HAP}$) are the same or similar. The correlation of pH and solution fluoride inhibitory effect on CAP solubility was also investigated for CAP P706 sample. As can be seen in Figure 6.13, the same pH effect as found for CAP P954 was observed for this CAP preparation.

To further understand the influence of solution fluoride on CAP solubility, one of these CAP samples, CAP P706, was selected for an in-depth study. The solubility distribution of sample CAP P706 is presented in Figure 6.14. In this study, experiments
Figure 6.12 The pattern of solution fluoride affecting the solubility of CAP P954 at pH 6.5 (O) and pH 5.0 (□). $p_{\text{I}_{\text{HAP}}}$ conditions are 117.0 for the pH 6.5 solutions and 116.7 for the pH 5.0 solutions.
Figure 6.13 The pattern of solution fluoride affecting the solubility of CAP P706 at pH 6.5 (O) and pH 5.0 (□). pH$_{HAP}$ conditions are 112.1 for the pH 6.5 solutions and 112.3 for the pH 5.0 solutions.
Figure 6.14 The solubility (MES) distribution of CAP P706. The experiments were conducted in partially saturated solutions (defluoridated) of pH 6.5. The best fit curve is based on equation $y = 50\{1+\text{erf}[k(x-x_0)]\}$, where \text{erf} is the error function.
were conducted to investigate the influence of solution fluoride on the CAP solubility at three solution pI_HAP conditions (pH 6.5). Again, as can be seen from Figure 6.15, solution fluoride affecting the CAP solubility is not abrupt but, instead, gradual at low solution fluoride levels. When fluoride concentrations are reduced less than about 0.02 ppb, the disturbance of solution fluoride on the CAP solubility is very small, and the CAP solubility behavior without the interference of solution fluoride can be studied in solutions under such conditions. In the previous discussion of CAP H956, which was synthesized by the hydrolysis method, it was reported that fluoride threshold concentrations were lower when the solution pI_HAP value was smaller. However, for CAP P706 which was synthesized by the precipitation method, different solution saturation levels (i.e., solution pI_HAP of 112.1, 112.7 and 113.2) seemed to have little influence on the pattern of fluoride inhibiting the CAP solubility. While the physicochemical base was not quite clear for the almost parallel fluoride effects on the CAP P706 solubility at three solution pI_HAP conditions, the response to solution fluoride could be different for CAP samples prepared by different synthesis methods.

Study of Solution Fluoride Affecting CAP P706 Solubility after Pre-equilibrating CAP with Solution Fluoride

Experiments on the dissolution of CAP in a powder suspension system (21, 22) have shown that it requires a period of time, from minutes to hours, for the CAP crystal to arrive at metastable equilibrium with the dissolution media. A calculation has also shown that there is required a similar period of time for the CAP powder to adsorb solution fluoride on the crystal surface. As a result of the parallel processes, part of the CAP solid
Figure 6.15 Threshold concentration of solution fluoride affecting the solubility of CAP P706 at pH 6.5. Values of solution $p\text{H}_{\text{HAP}}$ are 113.2 (O), 112.7 (□) and 112.1 (○). Free solution fluoride is plotted in linear in (a) and in logarithmic (b) fashion.
could dissolve before fluoride was adsorbed on the surface, and before the fluoride-containing surface complex entity began to govern the dissolution of the apatite. If this analysis is correct for apatite dissolution in fluoride containing media, under the same solution conditions, the CAP solubility in percent (MES determined by preequilibration with fluoride) will be less than that determined without preequilibration. To address this important question, the solubility of CAP P706 was measured after preequilibration treatment, with an objective to compare these results with those obtained without the preequilibration treatment.

The procedure to determine the CAP’s true solubility (MES), i.e., with the CAP preequilibrated in fluoride-containing dissolution media, was modified from the method without the preequilibration treatment. For determination of CAP solubility without preequilibration, as described before, CAP powder is equilibrated with partially saturated solutions under predetermined conditions (e.g., pH 6.5 and pI\textsubscript{HAP} 112.7) for 48 hours. To determinate CAP solubility with the preequilibration treatment, the pH of the dissolution media is first raised to a predetermined value (e.g., pH 6.8 and hence solution pI\textsubscript{HAP} becomes 109.6) such that the CAP does not dissolve in the solutions. After exposure for 24 h and the adsorption of fluoride on the crystal surface reaches a steady state, the pH of the dissolution media is adjusted back to the original level (e.g., pH 6.5 and solution pI\textsubscript{HAP} 112.7) so that the CAP starts to dissolve in the solutions. The rest of the experiment is exactly the same as the non-preequilibration procedure as describe before. In order to compare with the non preequilibration results of CAP P706 at pH 6.5 and three solution pI\textsubscript{HAP} conditions, the preequilibration experiments were conducted at the same solutions compositions and the results are shown in Figure 6.16a. Figure 6.16b presents the
Figure 6.16 CAP P706 solubility (MES). (a) CAP P706 true solubility (MES) (CAP preequilibrated with solution fluoride) versus solution fluoride concentration at pH 6.5. Values of solution pH$_{\text{HAP}}$ are 113.2 (O), 112.7 (□) and 112.1 (○). (b) Comparison of solution fluoride affecting CAP P706 solubility with (open symbols) and without (closed symbols) pre-equilibration treatment. Solution pH$_{\text{HAP}}$ are 113.2 (O, ●), 112.7 (□, ■) and 112.1 (○, ◆).
comparison of the CAP solubility versus solution fluoride concentration with and without preequilibration treatment. From the three sets of results, it is seen that the CAP solubility (MES) determined by the preequilibration method is slightly less than that determined by the standard method. This observation was consistent with the previous theoretical supposition. In addition, the small difference of the CAP "solubility" found by the two methods suggests that the solution fluoride ions are adsorbed faster than the rate of apatite dissolution in the present solution conditions (e.g., pH 6.5) so that fluoride adsorption is almost complete before a significant amount of the apatite is dissolved.

Because of the data scatter as given in Figure 6.15 and 6.16, some may consider that there was almost no fluoride effect on the CAP solubility at extremely low levels of solution fluoride (e.g., ≤ 0.02 ppb), others may argue there was a slightly measurable fluoride effect on the CAP solubility even at such low fluoride levels. Previous studies by Chhettry et al. (23) found that in the absence of solution fluoride, the solubility (MES) distributions of CAPs are controlled by a surface complex possessing the stoichiometry of HAP, Ca_{10}(PO_4)_6(OH)_2. It has also been shown by Zhuang et al. (24) that a surface complex with the stoichiometry of FAP, Ca_{10}(PO_4)_6F_2, best describes the solubility distributions of CAPs in the presence of high levels of solution fluoride and at low solution pH (e.g., pH 4.5). Therefore, it is quite reasonable that a surface complex with a stoichiometry of Ca_{10}(PO_4)_6(OH)_{2-x}F_x (0 ≤ x ≤ 2) may govern the solubility of CAPs at low solution fluoride concentrations that correspond to where fluoride begins to take part in the surface complex entity. To examine the sensitivity of solution fluoride affecting the solubility of the CAP based upon the experimental data that were scattered, the data presented in Figure 6.15 were fitted by different equations. In each case, the
stoichiometry $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-x}F_x$ was examined to find the value of $x$ for a particular solution fluoride level. The purpose of this data treatment was to figure out the asymptotic functional form (i.e., the value of $x$) governing the solubility of the CAP as solution fluoride was reduced to ultra-low levels (i.e., $\leq 0.02$ ppb).

The experimental data in Figure 6.15 was first fit by bell-shaped curves with an equation of $y = a \cdot \exp(-b \cdot x^2)$, where $y$ and $x$ represent the CAP percent dissolved and solution fluoride concentration, respectively, and parameters $a$ and $b$ were determined from data fitting. Such a fitting curve has a relatively flat (plateau) region when fluoride levels are very low (i.e., $\leq 0.02$ ppb) and this plot is shown in Figure 6.17a. The results of Figure 6.17a (i.e., the plots of percent CAP dissolved versus solution fluoride) were then transformed in Figure 6.18 to plots of percent CAP dissolved versus $-\log (\text{IAP})$ where IAP is the ion activity product for stoichiometry $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-x}F_x$. In Figure 6.18, two representative cases, (a) $x = 0.8$ and (b) $x = 1.5$, are shown. Where superposition of the curves take place, the corresponding $x$ value was the "correct" $x$ in that fluoride range. For example, for $x = 0.8$ in Figure 6.18a, curves #4 and #5 essentially superpose. Curves #4 and #5 correspond to 0.06 and 0.08 ppb solution fluoride, respectively. This means that in this fluoride concentration range, $x = 0.8$ is the correct value. In another example (Figure 6.18b), curves #6 and #7 correspond to 0.10 and 0.12 ppb solution fluoride, respectively, and this means that in this fluoride concentration range, $x = 1.5$ is the correct value. Figure 6.19a presents the results of the determined $x$-values over the fluoride concentration range, 0 to 0.15 ppb. As can be seen, as the fluoride concentration approaches zero, the $x$-value asymptotically approaches $x = 0$ where the surface complex stoichiometry becomes that of hydroxyapatite.
Figure 6.17 Relationship of CAP P706 solubility versus solution fluoride concentration at pH 6.5 and solution pHAP of 113.2 (O), 112.7 (□) and 112.1 (O) (the same data as shown in Figure 6.15). (a) Data are fit by a bell-shaped function $y = a \cdot \exp(-b \cdot x^2)$; (b) data are fit by a linear function $y = a \cdot x + b$; (c) data are fit by eye. Parameters $a$ and $b$ are determined from data fitting.
Figure 6.18 Solubility of CAP P706 (see Figure 6.15 and 6.17a) at pH 6.5 is plotted as a function of the negative logarithm of the ion activity product (IAP) with respect to Ca$_{10}$(PO$_4$)$_6$(OH)$_{2x}$F$_x$. (a) $x = 0.8$; (b) $x = 1.5$. 
Figure 6.19 The relationship between x and solution fluoride, where x occurs in the formula Ca_{10}(PO_4)_6(OH)_{2-x}F_x. Plot of (a) is based on a bell-shaped function fit (see Figure 6.16a); (b) is based on a linear function fit (see Figure 6.16b); and (c) is based on an eye-ball fit (see Figure 6.16(c)).
Let us now examine the experimental data in Figure 6.15 fit by linear curves with an equation of \( y = a \cdot x + b \), where \( y \) and \( x \) represent the CAP percent dissolved and solution fluoride concentration, respectively; and parameters \( a \) and \( b \) are determined from data fitting. Apparently, such a fitting curve does not have a plateau region when fluoride levels are very low (i.e., \( \leq 0.02 \) ppb) and this plot is shown in Figure 6.17b. With the same data transformation procedure as demonstrated in Figure 6.18, the results of the \( x \)-value versus fluoride concentration are shown in Figure 6.19b. Similarly, when the experimental data in Figure 6.15 were fit by eye which is given in Figure 6.17c, the obtained relationship between the \( x \)-value and fluoride concentration is presented in Figure 6.19c. Figure 6.19a, b, and c show that the relationship of \( x \)-value and fluoride concentration are similar or almost the same no matter which equation or curve is selected to fit the data shown in Figure 6.15. Therefore, the obtained relationship of the \( x \)-value and fluoride concentration is independent of the fitting curves and hence the data scattering, as shown in Figure 6.15, would not affect the conclusion that the fluoride effect became asymptotically negligible as solution fluoride is reduced to ultra-low levels, i.e., \( \leq 0.02 \) ppb.

As shown in Figure 6.16a, the dependence of CAP P706 solubility and solution fluoride concentration was also determined with the CAP pre-equilibrating with solution fluoride. When the data in Figure 6.16a were treated by the same method as used in the data treatment of Figure 6.15, similar results as that of Figure 6.19 (not shown) of the relationship of the \( x \)-value and fluoride concentration were obtained (with pre-equilibration treatment). Therefore, as solution fluoride is reduced to extremely-low levels, i.e., \( \leq 0.02 \) ppb, the functional form governing the CAP solubility asymptotically
approaches the stoichiometry of hydroxyapatite, which was consistent with the results reported by Chhettry et al. (21). Also, the effect of solution fluoride influencing the CAP solubility became asymptotically negligible at these low solution fluoride levels (i.e., \( \leq 0.02 \) ppb). In addition, at higher solution fluoride levels, the solubility governing function (stoichiometry of the surface complex entity) would increasingly involve the solution fluoride concentration.

**Application of the Threshold Solution Fluoride Study on CAP MES Research**

Partially saturated solutions have been used for solubility and dissolution studies on synthetic apatites as well as tooth and bone minerals. Since many reagents (such as CaCO\(_3\) and CaCl\(_2\)) contain trace amounts of fluoride impurity, the solutions prepared using these reagents can possess fluoride concentrations much higher than the threshold fluoride levels, and thus interfere with the dissolution and solubility studies of apatites. Figure 6.20 shows the MES distributions of CAP P956 determined in partially saturated solutions with and without removing the fluoride impurity from the dissolution media. The AR grade CaCO\(_3\) (Mallinckrodt, Inc., Paris, Kentucky) was used here as the source of solution calcium in the preparation of partially saturated solutions, and the solution fluoride concentration was found to be 15 – 20 ppb. When the CAP solubility was measured in such solutions without defluoridation, as can be seen in Figure 6.20, slurry densities (i.e., solid-to-solution ratios) affect the experimental results: the determined CAP solubility (MES) was higher in the high slurry density system (50mg/250ml, curve 2) than that measured in low slurry density system (10mg/250ml, curve 1). This indicates that the solution fluoride inhibitory effect is reduced as the slurry density is increased. Such an
Figure 6.20 Metastable equilibrium solubility (MES) distributions of CAP P956 determined with partially saturated solutions defluoridated (open symbols) and nondefluoridated (closed symbols). Slurry densities (ratios of amount of CAP used to solution volume) in the experiments were: 10mg/1000ml (○), 10mg/250ml (O, ●), and 50mg/250ml (□, ■). Best fitting curves are obtained based on equation $y = 50\{1 + \text{erf}[k(x-x_0)]\}$, where erf is the error function.
observation can be explained: in the high slurry density system, the CAP residue can adsorb more fluoride ions on the apatite surface. As a result, the free solution fluoride concentration decreases (but still remains higher than the threshold level), and therefore the CAP solubility is less inhibited by solution fluoride.

When partially saturated solutions were defluoridated, as shown in Figure 6.20, the CAP MES distributions determined at three different slurry densities (10mg/1000ml, 10mg/250ml and 50mg/250ml) are essentially superposed to curve 3 within experimental error. The independence of CAP solubility on the slurry density demonstrates that the fluoride inhibitory effect on the CAP solubility was practically negligible in these defluoridated solution systems, and the fluoride concentrations in these solutions were below the threshold level. Apparently, the MES distribution profile determined in the defluoridated solution systems represents the true solubility behavior of the CAP in the absence of solution fluoride.

The results discussed above show that the solution fluoride inhibitory effect on apatite solubility can be dramatic if fluoride impurity in the reagents is not removed. Therefore, without knowing the solution fluoride concentrations in the dissolution media, the validity of the results of apatite solubility studies can be greatly compromised due to the fluoride effect. Furthermore, the present findings can be used to interpret the earlier investigations of dental enamel subsurface demineralization (11). In the presence of solution fluoride, while the surface of enamel is exposed to solution fluoride, the subsurface of enamel is exposed to ambient solution without fluoride (or fluoride levels below the threshold). From the results of solution fluoride inhibitory research, the dissolution of surface layer of enamel could be totally inhibited because of the solution
fluoride. However, the subsurface of enamel could be dissolved because the fluoride inhibitory effect does not exist there (where fluoride concentration is below the threshold level and the dissolution follows $\text{IAP}_{\text{HAP}}$ surface complex).

Although many significant achievements in understanding the solubility behavior of apatites have been made by this research group in the past several decades, there yet remain many questions to be answered. As discussed in the previous paragraphs, the slurry density effect in determining CAP H956 solubility was eliminated when the dissolution media were defluoridated. However, in the study of CAP P954 solubility (MES) properties in the absence of solution fluoride, it was found that the CAP MES varied with slurry density even at defluoridated solutions (see Table 6.10). Five sets of slurry density effect experiments were done with three methods to defluoridate the solutions. Since the present technique was unable to reduce the solution fluoride concentrations to absolute zero, trace amounts of fluoride present in the defluoridated solutions could possibly cause the inhibitory effect on the CAP solubility. Therefore, the solution fluoride concentrations were measured before and after the CAP equilibration with the solutions. As can be seen in Table 6.10, for each test set, although the high slurry density experiment is likely to produce a higher percentage of CAP solubility (represented in bold), the corresponding solution fluoride level(s) after the equilibration period is always lower than those in low slurry density (represented in italic). Therefore, although the exact reasons accounting for the slurry density effect of CAP P954 is not clear yet, solution fluoride effect is one, if not the only of the important factors that cause the CAP solubility to vary with slurry density.
Table 6.10 Slurry density effect on CAP P954 solubility (MES) and solution fluoride concentrations before and after the equilibration period for MES determination. The solution volume in each CAP MES determination was 2 liters.

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Defluoridation Method</th>
<th>Solution pH&lt;sub&gt;HAP&lt;/sub&gt;</th>
<th>Solution F before MES</th>
<th>CAP percent dissolved and solution F after MES</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>[CAP used 80 mg]</td>
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<td>[CAP used 20 mg]</td>
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<td></td>
<td></td>
<td></td>
<td>[CAP used 10 mg]</td>
</tr>
<tr>
<td>1</td>
<td>RE**</td>
<td>116.7</td>
<td>0.40 ppb</td>
<td>52 %</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.07 ppb</td>
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<td>34 %</td>
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<td>0.10 ppb</td>
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<td>11 %</td>
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<td></td>
<td></td>
<td></td>
<td>0.31 ppb</td>
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<tr>
<td>2</td>
<td>RE**</td>
<td>116.2</td>
<td>0.48 ppb</td>
<td>22 %</td>
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<td>0.07 ppb</td>
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<td>8 %</td>
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<td>0.12 ppb</td>
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<td>8 %</td>
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<td>0.29 ppb</td>
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<tr>
<td>3</td>
<td>UC***</td>
<td>116.5</td>
<td>0.09 ppb</td>
<td>81 %</td>
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<td>0.08 ppb</td>
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<td>64 %</td>
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<td>0.09 ppb</td>
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<td></td>
<td>52 %</td>
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<td></td>
<td></td>
<td>0.12 ppb</td>
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<tr>
<td>4</td>
<td>UC***</td>
<td>116.5</td>
<td>0.18 ppb</td>
<td>69 %</td>
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<td></td>
<td>0.06 ppb</td>
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<td>63 %</td>
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<td>0.06 ppb</td>
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<td>26 %</td>
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<td></td>
<td></td>
<td>0.09 ppb</td>
</tr>
<tr>
<td>5</td>
<td>SE****</td>
<td>116.4</td>
<td>0.15 ppb</td>
<td>59 %</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>0.06 ppb</td>
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<td></td>
<td>66 %</td>
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<td>0.07 ppb</td>
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<td></td>
<td></td>
<td>0.09 ppb</td>
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</tbody>
</table>

* Data from Anil Chhettry.

** RE: the stock solutions were defluoridated by using HAP twice.

*** UC: RE method plus the dissolution media were defluoridated by using HAP once and CAP once.

**** SE: RE method plus the dissolution media were defluoridated by using HAP twice.
Conclusions

In this chapter, systematic studies on solution fluoride affecting the CAP solubility (MES) at ultra-low levels has been discussed. The main findings and results of this research can be generalized as:

1. A procedure has been developed and improved which permits the determination of fluoride concentrations as low as 0.02 ppb in both water and dissolution media.

2. A method has been developed and validated which can reduce fluoride levels in partially saturated solutions down to 0.04 to 0.1 ppb.

3. The threshold concentrations of solution fluoride influencing CAP solubility are at sub-ppb levels. The threshold concentrations may be different for CAP samples prepared by different synthesis method and under different synthesis conditions.

4. For the same CAP preparation, solution fluoride has more inhibitory effect on the CAP solubility in solutions at a lower pH (e.g., pH 5.0) than that at a higher pH (e.g., pH 6.5). In other words, the lowest (threshold) fluoride concentration influencing the CAP MES behavior is less in lower pH solutions than that in higher pH solutions.

5. With the three CAP samples synthesized by the precipitation method, the influence of solution fluoride on the CAP solubility was not abrupt but, instead, was gradual: the fluoride effect became asymptotically negligible as solution fluoride was reduced to ultra-low levels, i.e., ≤ 0.02 ppb.

6. At low solution fluoride levels (i.e., ≤ 0.02 ppb) the solubility governing function is consistent with a surface complex possessing hydroxyapatite stoichiometry.

7. At higher solution fluoride levels, the solubility governing function (surface complex entity) increasingly involves the solution fluoride concentration.
8. Solution fluoride impurity is one of the important factors causing the slurry density effect on the determination of CAP solubility (MES) in the absence of solution fluoride.
References


CHAPTER 7

SUMMARY

The solubility behavior of carbonated apatites in the presence of solution fluoride has been studied in this dissertation. The main projects involved in this study are: whether CAPs exhibit metastable equilibrium solubility phenomena in the presence of solution fluoride and what is the surface complex stoichiometry governing the CAP solubility, relationships involving the CAPs solubility, surface complexes and crystallite disorder, and the lowest (threshold) concentrations of solution fluoride affecting the solubility behavior of the CAPs.

In Chapter 4, the investigation were to assess the applicability of the metastable equilibrium solubility (MES) concept for the carbonated apatites (CAPs) over a range of pH and a wide range of solution fluoride concentrations and to examine the hypothesis that, in the presence of solution fluoride, a surface complex with the stoichiometry of fluorapatite (FAP) governs the MES behavior. Two CAP samples were prepared by precipitation from reaction media containing calcium nitrate (Ca(NO$_3$)$_2$·4H$_2$O) and sodium phosphate (NaH$_2$PO$_4$·H$_2$O) at two different levels of sodium bicarbonate. The MES distributions were determined with the two CAP preparations by equilibrating approximately 10 mg of CAP powder in 2 liters of 0.1 M acetate buffers (ionic strength = 0.50 M) at pH 4.5 and 5.5 and at various levels of calcium, phosphate and fluoride. The fluoride concentrations ranged from 0.03 to 12 ppm. From the compositions of the
equilibrating buffer solutions, ion activity products based upon the stoichiometries of hydroxyapatite (HAP) and FAP were calculated in an attempt to determine the correct function governing the dissolution of the CAP preparations. The results of this study demonstrated that both CAP preparations exhibit the MES distribution phenomenon in solution media of varying pH and fluoride concentrations. Furthermore, the experimental MES data obtained with both CAP preparations at the lower pH (4.5) and at higher solution fluoride levels ($\geq 0.1$ ppm) were essentially superimposable when plotted against the ion activity product based upon the stoichiometry of FAP, suggesting that in the presence of solution fluoride the MES governing surface complex may be entity possessing a stoichiometry approximated by that of FAP. When the HAP stoichiometry was assumed to represent the surface complex, good superposition of the data was not possible.

Previous studies have shown that the MES behavior of CAPs may be described by a surface complex with the stoichiometry of HAP in the absence of solution fluoride. In Chapter 4, results have shown that a surface complex with the stoichiometry of FAP governs the CAP MES when appreciable solution fluoride is present. Previous studies have also shown that the magnitude of the MES is determined by the crystallinity of the CAPs. The aims of the investigation in Chapter 5 were to examine the relationship between CAP MES determined in the presence of solution fluoride and crystallinity and to address the question of the effect of the change in the stoichiometry of the surface complex (from HAP to FAP) upon the relationship of the MES to crystallinity. The CAP samples were prepared by two methods: Method A involved precipitation of calcium nitrate and sodium phosphate solutions at constant pH at temperatures of 70, 85, and
95°C; Method B involved the hydrolysis of dicalcium phosphate dihydrate (DCPD) in bicarbonate containing solutions at 50, 70, and 95°C. The MES of CAPs was determined by equilibrating small amounts of powdered samples for 48 hours in a series of partially saturated 0.1 M acetate buffer solutions at pH of 4.5 at different fluoride concentrations at 30°C. From x-ray diffraction experiments, the crystallite microstrain and the full width at half maximum (FWHM) of the 002 reflection were determined and used as the crystallinity parameters for the CAPs synthesized by Method A and Method B, respectively. The plot of the mean MES (i.e., mean $pK_{FAP}$) values versus the crystallinity parameter (i.e., microstrain and FWHM) yielded a linear relationship with a slope essentially the same as that obtained in the absence of solution $F^{-}$ (i.e., when the mean $pK_{HAP}$ values were plotted against the crystallinity parameter). This parallel relationship between the MES and crystallinity in the absence (i.e., HAP surface complex) and the presence (i.e., FAP surface complex) of solution $F^{-}$ suggests that the degree of the CAP crystallite disorder affects the energetics of the two surface complexes (FAP and HAP) essentially to the same extent over a wide range of crystallinities, and this provides new insight into the nature of CAP surface complexes.

Solution fluoride has a dramatic influence on the solubility behavior and dissolution kinetics of apatites. In preliminary experiments, it was found that fluoride concentrations at ppb levels could influence the results of solubility studies of carbonated apatites. Also, fluoride is strongly adsorbed onto the surfaces of apatite crystal during the solubility or dissolution experiments, and it is the free solution fluoride that affects the solubility or dissolution of apatitic minerals. The aims of studies in Chapter 6 has been to assess the threshold fluoride concentrations that correspond to where fluoride begins to
have an inhibiting effect upon carbonated apatite solubility and to examine the nature of the mechanism of fluoride participation in the solubility reduction. In this research, a method has been developed to use CAP as an adsorbent to extract and concentrate solution fluoride by more than 1000-fold and be able to determine solution fluoride concentrations as low as 0.02 ppb. Also, a procedure removing fluoride (defluoridation) from dissolution media has been developed and evaluated, which can reduce solution fluoride levels down to about 0.04 to 0.1 ppb. CAP samples over a range of crystallinities were synthesized by the precipitation method at 70, 85 and 95 °C. In addition, a CAP sample prepared by the hydrolysis method at 95 °C was also used in this study. The dissolution media were prepared and defluoridated with the ion activity products determined in acetate buffers at predetermined pH an then known amounts of fluoride were added to these solutions. After equilibration the CAP samples in dissolution media with or without the added levels of fluoride, the undissolved residues were analyzed to determine the amounts dissolved and the supernatant fluoride concentrations were determined. This study has found that the sub-ppb levels of solution fluoride can repress the solubility of CAPs and solution fluoride has a stronger inhibitory effect on the CAP solubility when the pH of the solution is low. For the CAP samples prepared by the precipitation method, it has been found that the influence of solution fluoride concentration upon the CAP solubility was not abrupt but, instead, was gradual: the fluoride effect became asymptotically negligible as solution fluoride was reduced to ultra-low levels, i.e., ≤ 0.02 ppb. At these low solution fluoride levels (i.e., ≤ 0.02 ppb) the solubility governing function is consistent with surface complex possessing the hydroxyapatite stoichiometry, which agrees with the earlier findings of the CAP research.
in this laboratory. At higher solution fluoride levels, the solubility governing function (stoichiometry of surface complex entity) increasingly involves the solution fluoride concentration.