

# Hydroxyapatite Dissolution Rates in Fluoride/Dodecylamine Solutions<sup>1</sup>

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The influence of fluoride (F) alone and together with dodecylamine hydrochloride (DAC) on the dissolution rate behavior of hydroxyapatite (HAP) crystal suspensions has been studied. The results showed that F and DAC inhibit the dissolution in an additive rather than a synergistic manner. The data suggest that DAC inhibits dissolution from the predominant surface(s) by adsorption physically blocking the HAP surface while F inhibits the same surface by influencing the apparent driving force for dissolution. The existence of a residual component for the dissolution rate at high concentrations that is about 15% of the maximum rate suggests that there are two or more dissolution sites on the HAP crystal. The one associated with the predominant surface(s) is strongly inhibited by both F and DAC whereas the other site(s) is insensitive to F either intrinsically or, in the presence of DAC, because of possible antagonism.

## INTRODUCTION

Fluoride (F) effects upon dissolution of enamel and hydroxyapatite (HAP) have long been subjects of interest and have been studied extensively by many investigators (1, 2). Fluoride is being used prophylactically as an anticaries agent and in some instances, the combination of fluoride and a long-chain alkyl amine has been used (3). A review of literature reveals, however, that very few studies have focused on combined action of an alkyl amine with fluoride from the mechanistic point of view. The purpose of this study was, therefore, to investigate the influence of F alone and together with a long-chain alkyl amine, dodecylamine hydrochloride (DAC), on the dissolution behavior of HAP and to correlate the data obtained with DAC adsorption onto HAP. The effect of DAC alone has been reported in an earlier study (4). This investigation was conducted by using a well-defined HAP suspension method which has been demonstrated previously (5) to involve essen-

tially 100% surface-controlled kinetics under appropriate conditions.

## MATERIALS

*Hydroxyapatite.* Samples used in this study were labeled as UM-M, Y146, and Y155.

Both Y146 and Y155 were provided by Dr. R. A. Young of Georgia Institute of Technology. Y155 was hydrothermally prepared in two steps. In the first step of this synthesis, a solid-state reaction between CaO and Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub> was carried out at 1000°C. The resulting powder was then ground and loaded into a platinum-lined hydrothermal bomb with water, and held at 500°C, 15,000 psi for 1 month.

Y146 was prepared by refluxing dicalcium phosphate dihydrate in distilled and deionized water for 1 month.

UM-M was synthesized in this laboratory by a method which was a slight modification of the one described by Avnimelech *et al.* (6). Pure phosphoric acid was added at a slow controlled rate to a boiling, carbon dioxide-free, calcium oxide solution in a Teflon flask. The resulting precipitate was digested for 2 weeks

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in the boiling-reaction solution with constant bubbling  $N_2$  ( $CO_2$  free). The residue was centrifuged and the supernatant liquid was decanted. Freshly boiled double-distilled water was then added, the suspension was stirred and centrifuged again. This same procedure was repeated three times. The final residue was then dried at  $110^\circ C$  overnight.

The chemical composition, specific surface area, and major impurities of the samples are given in Table I.

*Dodecylamine hydrochloride (DAC)*. Reagent grade DAC was obtained from Eastman-Kodak Company. It was then recrystallized once in benzene. The purity was verified by means of paper chromatography.

*Solutions*. Calculated amounts of an acetic acid and a sodium acetate stock solution were used to make up the buffer solution of desired pH which, in this study, was 4.50. DAC and fluoride solutions were made by dissolving predetermined amounts of DAC and sodium fluoride in the buffer. Sodium chloride was

added to all buffer solutions to maintain the ionic strength of 0.1. The pH of the solutions was measured to an accuracy of  $\pm 0.01$  pH unit using an Altex Model 4500 digital pH meter. Reagent grade chemicals were used in all preparations.

## METHODS

### *Experimental Methods*

*Dissolution*. Hydroxyapatite in clumps was broken down by light grinding until no visible lumps were present. Exactly 10 mg of HAP was suspended in 12.5 ml of double-distilled water in a 50-ml water-jacketed ( $30^\circ C$ ) reaction vessel. This suspension was magnetically stirred for about 20 sec at 600 rpm before being sonified (ultrasonic generator Model AK500, Acoustica, Los Angeles, Calif.) for 60 sec to produce a milky suspension. After sonication, stirring was resumed and 12.5 ml of dissolution medium (double concentration) was added to the suspension. Addition and mixing was completed in 1 to 2 sec, and the pH at this point was recorded to be  $4.50 \pm 0.01$ . Samples of 0.5 ml were withdrawn at predetermined times by an Eppendorf pipet during the dissolution experiment and immediately passed through a Millipore filter paper (GSU) with a pore size of  $0.22 \mu m$ . Sampling and filtering was completed within 4 to 6 sec. The filtrate was analyzed for phosphate and/or calcium.

*Adsorption*. HAP (500 mg) was quantitatively transferred to a 25-ml water-jacketed ( $30^\circ C$ ) vessel containing 10 ml of DAC solution. The suspension was sonified (ultrasonic generator Model AK500, Acoustica) for 3 min before being magnetically stirred at 600 rpm. The system was kept closed throughout the experiment to avoid evaporation. At adsorption equilibrium, samples withdrawn by an Eppendorf pipet were then passed through a Millipore filter paper (GSU) with a pore size of  $0.22 \mu m$ . The filtrate was analyzed for phosphate and dodecylamine hydrochloride.

TABLE I

Chemical Composition, Specific Surface Area, and Major Impurities of Different Hydroxyapatite Samples

HAP	Calcium <sup>a</sup> (wt%)	Phosphate <sup>a</sup> (wt%)	Ca/P <sup>a</sup> (M)	S.A. <sup>b</sup> (m <sup>2</sup> /g)	Major impurity
NBS-P	40.00	55.31	1.714	22.5	None
NBS-B	39.76	53.83	1.644	—	None
UM-T	39.20	55.83	1.664	—	0.3% $CO_3^c$
UM-K	40.20	55.00	1.732	22.9	0.3% $SiO_2$
UM-A	39.49	54.94	1.703	23.4	0.2% $CO_3^c$
UM-M	39.37	55.14	1.692	23.0	None
Y146	—	—	1.60	17.3	$HPO_4^c$
Y155	—	—	1.65	2.3	None
Bio-Gel	—	—	—	69.8	Not analyzed

<sup>a</sup> The concentrations of calcium and phosphate and Ca/P molar ratio were determined by General Electric Co. (Cleveland, Ohio); calcium by an EDTA volumetric method and phosphate by a triple-precipitation gravimetric method.

<sup>b</sup> The specific surface area which was determined by Micromeritics (Norcross, Georgia) based on BET calculation using nitrogen adsorption.

<sup>c</sup> Estimated from IR spectra.

### *Analytical Methods*

*Phosphate.* Phosphate concentrations were determined according to the method of Gee *et al.* (7). The phospho-ammonium molybdate complex formed was reduced by stannous chloride. The absorbance of the resulting color was determined after 15 min at  $\lambda = 720$  nm in a Beckman Model 25 spectrophotometer.

*Dodecylamine hydrochloride.* DAC concentrations were determined according to the method of Ino *et al.* (8) with slight modifications. This antagonistic titration used sodium lauryl sulfate as the titrant and rhodamine 6G as the adsorption indicator. The simultaneous formation of a pink precipitate and color change, with loss of fluorescence, was taken to be the equivalent point.

## RESULTS

### *Effect of Fluoride on Dissolution of Hydroxyapatite*

Pretreatment of the sonicated HAP powder with F for various lengths of time ranging from 5 to 30 min prior to conducting dissolution rate experiments provided no additional inhibition against dissolution indicating that the action of F is extremely fast. Since the pretreatment step was unimportant, it was omitted in the standard procedure.

Figure 1 presents the raw data for the dissolution of three HAP preparations in acidic buffer (0.1 M acetate buffer, pH 4.50,  $u = 0.1$ ) containing various levels of F. Sample UM-M showed a significant F dependence up to 20 ppmF. As shown in this figure, increasing the F levels gave rise to corresponding decreases of dissolution rate. The rate at 20 ppm was about 20% of the control. Further increase of F beyond this level (e.g., 50 and 100 ppm) resulted in little change. The rates, within experimental errors, were not distinguishable from the 20-ppm case. There was a sudden increase in the dissolution rate when the F level was increased to 200 or 500 ppm. This was probably due to the precipitation of cal-

cium fluoride liberating phosphate into the bulk solution. Sample Y155, despite its much slower kinetics in comparison with sample UM-M, also exhibited the same kind of F dependence. F lowered the dissolution rate of sample Y146 but, in contrast to UM-M and Y155, the initial rates were relatively independent of F beyond the lowest F concentration tested (i.e., 0.10 ppm). Within experimental error, concentrations of 0.1, 5, 10, and 100 ppm F, which represented a range of 1000-fold difference, all reduced the rate by around 50% for sample Y-146.

### *Effect of Fluoride together with DAC on Dissolution of Hydroxyapatite*

The first set of experiments was conducted by preequilibrating the HAP powder with either F or DAC for 300 sec before adding the other. In both cases, the results were not different from those obtained without preequilibration. The concentrations of F used in this study were 1, 10, and 20 ppm and, for DAC, 1.5, 2.5, and 3.5 mM were chosen which represented small and large amounts of adsorption on the HAP surface. These two additives were mixed together in various combinations.

Some typical raw data for the dissolution of UM-M in medium containing constant F concentration in combination with varying level of DAC are presented in Fig. 2. The initial dissolution rates, estimated from the initial linear regions, are plotted as functions of the DAC and F concentrations in the media in Figs. 3 and 4, respectively. As shown in Fig. 3, the presence of F did not qualitatively change the pattern observed with zero F, i.e., the largest decrease in rate still occurred between 1.5 and 2.5 mM DAC. However, the differences in the dissolution rates between 1.5 and 2.5 mM DAC decreased with increasing F in the medium and, at 20 ppm F, the influence of DAC was very small. Figure 4 shows that the 1.5-mM DAC curve is more or less parallel to the control. The principal reduction in dissolution rate occurred between

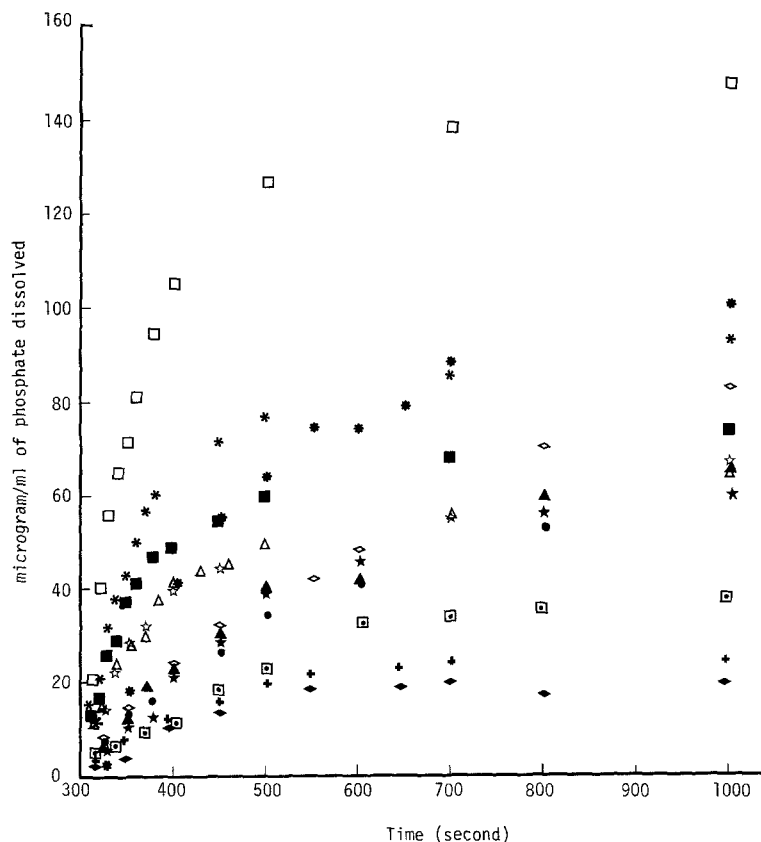


FIG. 1. Dissolution of HAP in 0.10 M acetate buffer, pH 4.50,  $\mu = 0.1$  at 30°C.

ppm F	UM-M	Y155	Y146
0	□	□	*
0.1			◇
1	*	+	★
10	■	◆	▲
20	△		●
100	☆		●

0 and 1 ppm F. For the 2.5- and 3.5-mM curves, the rates are about the same with or without F and equals about 15% of the control at 3.5 mM DAC.

#### Adsorption

Adsorption studies required large amounts of HAP powder. Since most samples were in limited supplies, only UM-M was included in the present work. Figure 5 shows the effect of

F on the adsorption of DAC onto HAP. The presence of F apparently did not alter the shape of the adsorption isotherms. They were all sigmoidal in nature with little adsorption until the equilibrium concentration reached 1.5 mM. The isotherms with F were displaced slightly to higher surfactant concentrations compared to the control (without F). The shifts were more pronounced at the higher equilibrium DAC concentration but the max-

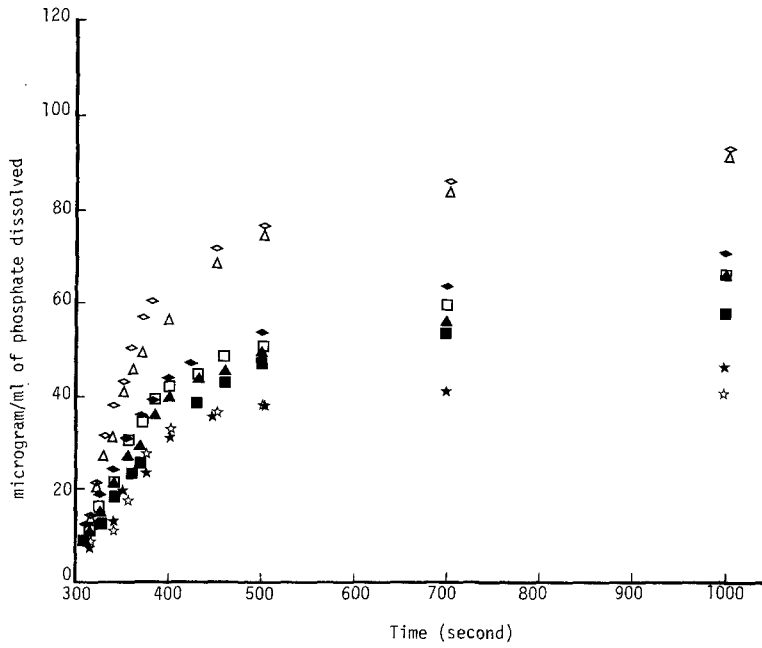


FIG. 2. Dissolution of HAP (UM-M) in 0.10 M acetate buffer, pH 4.50,  $\mu = 0.1$  at 30°C.

mM DAC	1 ppm F	20 ppm F
0	◇	◆
1.5	△	▲
2.5	□	■
3.5	☆	★

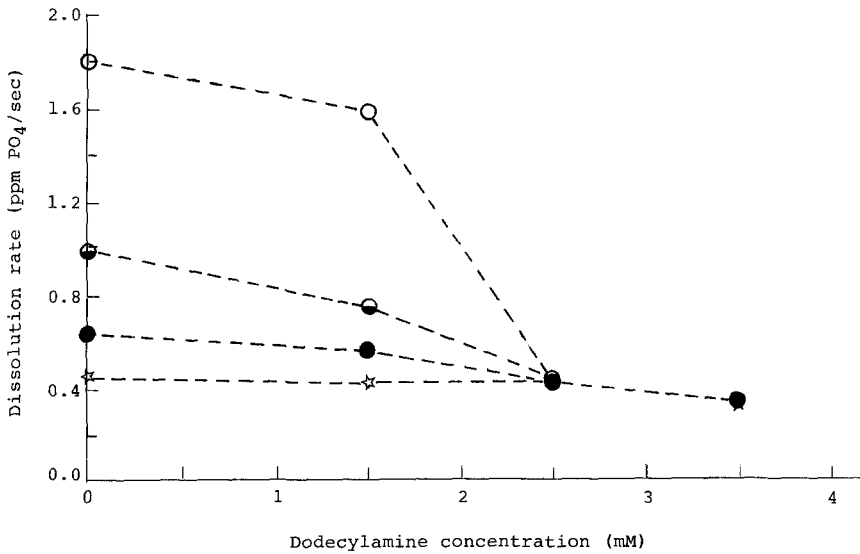


FIG. 3. Dissolution rate,  $J$ , as function of DAC in the medium for sample UM-M. F (ppm): ○, 0; ●, 1; ●, 10; ☆, 20.

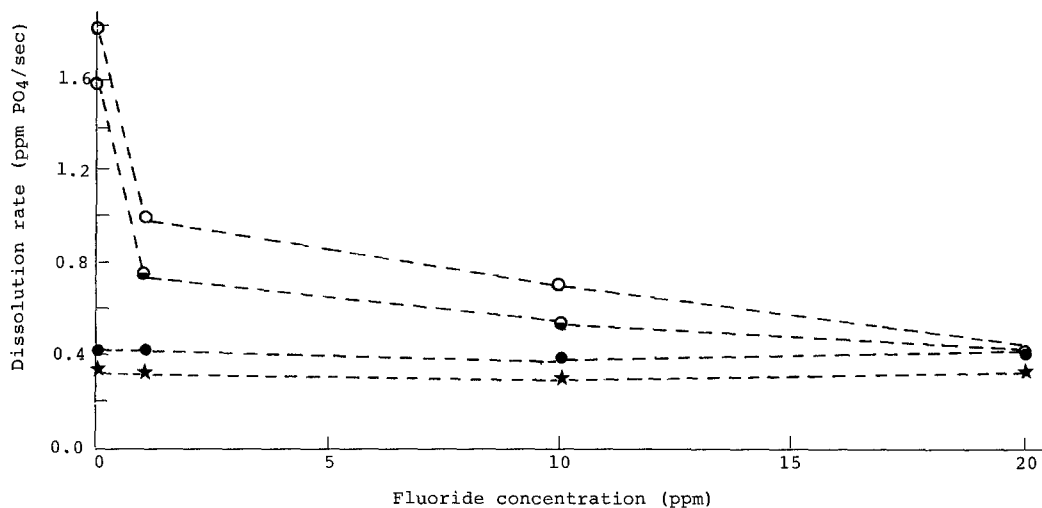


FIG. 4. Dissolution rate,  $J$ , as function of  $F$  in the medium for sample UM-M. DAC (mM):  $\circ$ , 0;  $\bullet$ , 1.5;  $\bullet$ , 2.5;  $\star$ , 3.5.

imum displacement was no more than 0.3 mM. The differences, if any, between isotherms of 5 and 20 ppm F were quite small. In fact, below 2 mM DAC, the data overlapped

considerably. Maximum adsorption occurred at about 3 mM DAC in all cases. The maximum amounts adsorbed remained the same as the control and was around 0.22 mM/gm.

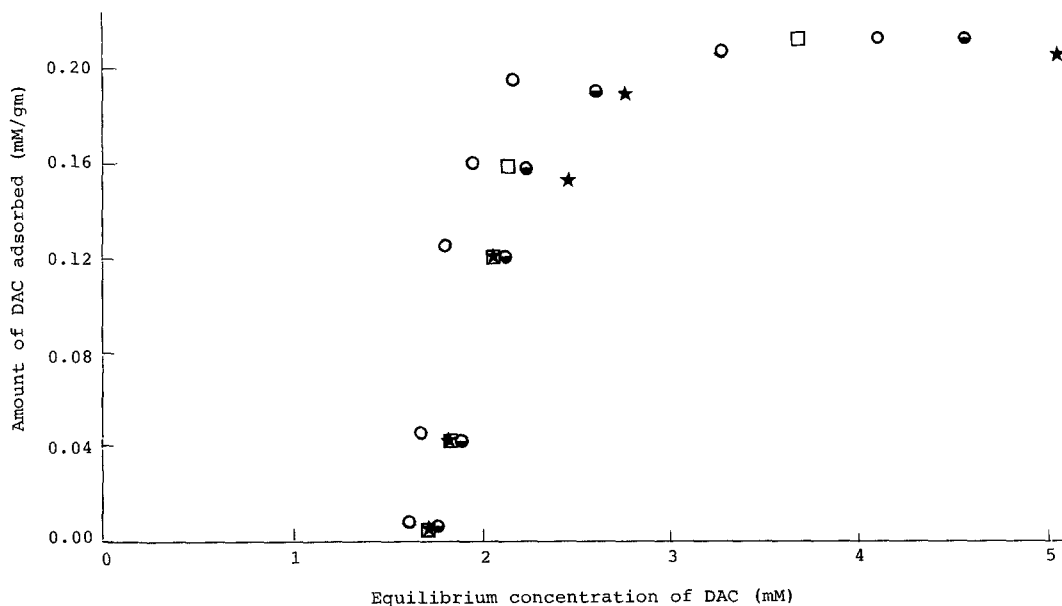
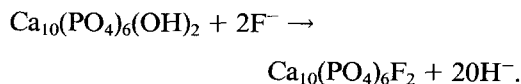


FIG. 5. Adsorption isotherms of DAC on HAP (UM-M) in 0.1 M acetate buffer, pH 4.50,  $\mu = 0.1$  at 30°C. F (ppm):  $\circ$ , 0;  $\square$ , 1;  $\bullet$ , 10;  $\star$ , 20.

## DISCUSSION

An explanation of the influence of F upon the dissolution rate behavior of HAP had been proposed earlier by Higuchi and his co-workers based on a "Fluorapatite" model (1). The key assumption in this model is that when HAP is exposed to a low concentration of F in acidic media, a thin layer of fluorapatite or a surface complex is formed around the HAP crystals by the reaction:



Such a layer may form rapidly by the isomorphous substitution of surface hydroxyls by F. A further assumption is that this FAP layer and not HAP is rate-governing during dissolution. Analysis of the UM-M and Y155 data with this model indicates that this mechanism may apply for these HAP samples. Table II shows the experimental results and the theoretical FAP model prediction. The reasonably good agreement between the experimental and theoretical values strongly suggests that the dissolution in the presence of solution  $\text{F}^-$  is indeed governed by a "FAP phase." Data obtained by Mir (1) and Dedhiya (9) using

TABLE II

The Ratio of HAP Dissolution Rate at 10 ppm F to that of 1 ppm F ( $J_{10}/J_1$ ) Obtained Experimentally and Theoretically Based on "Fluorapatite" Model

HAP	Theoretical $J_{10}/J_1^a$	Experimental $J_{10}/J_1$	
		0 mM DAC	1.5 mM DAC
UM-M	74.9	73	74
Y155	74.9	76	—
TVA <sup>b</sup>	74.9	77	—
TVA <sup>c</sup>	74.9	74	—

<sup>a</sup>  $J_{10}$  and  $J_1$  are the dissolution rates at 10 and 1 ppm F respectively; theoretical ratio obtained from FAP model.

<sup>b</sup> Data from Mir, N.A., Ph.D. thesis, The University of Michigan, 1967.

<sup>c</sup> Data from Dedhiya, M.G., Ph.D. thesis, The University of Michigan, 1978.

another synthetic HAP preparation (a high-temperature synthesis) are also listed in this table for comparison. Interestingly, at 1.5 mM DAC, the dissolution rate data for UM-M can also be fitted satisfactorily with the FAP model.

The rapid action of F suggests that the formation of FAP complex is extremely fast; this is consistent with the findings of Tung *et al.* (10). The irregular response of sample Y146 toward F may be related to its high  $\text{HPO}_4$  content. However, more work is required to elucidate the role played by  $\text{HPO}_4$ .

In contrast to the manner in which F inhibits the HAP dissolution rate, DAC appears to affect the HAP dissolution by a totally different mechanism. A previous study (4) showed that there is a correlation between the adsorption isotherm for DAC on HAP and the decrease in HAP dissolution rates as a result of the DAC adsorption. The correlation suggests that the primary mechanism for the dissolution inhibitive action of DAC is simply physical blocking of the surface effectively sealing it off from the ambient solution. These two inhibitors, DAC and F, appear to react with the predominant surface of HAP almost instantaneously by different modes of action. Birkeland and Rolla (11) have shown that the adsorption of protein and protein-dextran did not affect the F-HAP interaction to any great extent. If a layer of FAP complexes is formed on the surface regardless of the presence of DAC, the adsorptive properties of this layer with respect to DAC may not differ much from that of HAP alone. The unimportance of the order of letting the two inhibitors interact with the HAP and the only slight shift of adsorption isotherm both support this view. Regardless of the nature of the surface layer, once it is fully covered by the DAC, dissolution appears to be substantially shut off. The F then can exert its inhibitive effect only on the exposed surface, i.e., the magnitude of the F influence depends on how much surface is not covered by DAC. This proposed mechanism is consistent with the observation that generally F is more effective at zero or low DAC

concentration. For example, at 1.5 mM DAC when there is very little adsorption as shown by the isotherm (Fig. 5), 1 ppm F reduced the dissolution rate by about 50%, but at 2.5 mM DAC where the coverage is believed to be close to complete, none or very little F action is seen. This is clearly shown in Fig. 4. Figure 3 shows that, qualitatively, the curves of dissolution rate versus DAC concentration at varying levels of F are more or less the same. The largest drop in the rate is between 1.5 and 2.5 mM and this corresponds to the rapidly increasing region for adsorption. Thus there is an additive effect with these two inhibitors, but not a synergistic effect as one might have expected. Higuchi *et al.* (5) have shown that dissolution of HAP may be described by a first-order expression,

$$\text{Rate} = k(Cs - C)$$

where  $k$  is the first-order rate constant,  $C_s$  is the apparent solubility or the characteristic driving force governing the dissolution, and  $C$  is the microenvironmental solution concentration. From the above discussion, we may suggest that DAC's action is primarily to reduce  $k$  by physically blocking the surface whereas F lowers  $C_s$  by changing the governing "phase" from HAP to FAP.

There exists a residual dissolution rate which is about 15% of the maximum rate and is affected only very little by the addition of DAC in the solution beyond 3.5 mM. This residue might be related to the crystal surface not being completely covered by DAC at maximum adsorption (plateau region) and/or to the idea that another site(s) is present that is insensitive to DAC. Incomplete coverage may in part be related to the formation of micelles as the critical micelle concentration (CMC) is around 3.5 mM (4). Whatever the cause(s) may be, this residue is shown to be different from the component of the dissolution rate for the predominant surface since F does not have any effect on the former (see Fig. 4, 2.5 and 3.5 mM DAC cases). One might

argue that the F inhibition effects may still exist for this residual component but that the effects are coincidentally offset by increasing DAC desorption with increasing addition of F. This argument would be weak as the influence of F upon the DAC adsorption isotherm is small and the rate versus DAC concentration curve is flat in the region 2.5 to 3.5 mM (4). One might therefore conclude that there are two or more dissolution sites on the HAP crystal. Because of the observed correlation between DAC adsorption and dissolution inhibition (4), it may be presumed that one of these sites is strongly inhibited by both DAC and F in an additive fashion and it may be associated with the predominant surfaces of HAP (the prism planes). We now suggest that there is a second site which is insensitive to F (as far as *initial* dissolution rates are concerned) either intrinsically or because of possible DAC antagonism. The second site may also be relatively insensitive to DAC but this is difficult to determine because the solution DAC concentration is close to the CMC.<sup>2</sup> The existence of more than one site on HAP crystal is not unreasonable since work done in these laboratories consistently point to this phenomena (13).

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<sup>2</sup> It should be pointed out that a hydroxyapatite prepared at 1200°C (and designated as TVA hydroxyapatite) showed no significant residual initial dissolution in the plateau region of the DAC adsorption isotherm (see Ref. (12)). This suggests simultaneously that TVA hydroxyapatite obeys a "one-site" mechanism with regard to DAC inhibition of hydroxyapatite dissolution kinetics, that the residual dissolution rates observed in the present studies at high DAC levels is probably not caused by incomplete coverage arising from the CMC phenomenon, and that the adsorption layer (at saturation adsorption) is probably not sufficiently permeable to permit dissolution to proceed at a significant rate (because, for example, thermal fluctuations are inadequate).



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