

Quantitation of Enamel Demineralization Mechanisms II

Correlation of Synthetic Hydroxyapatite Initial Demineralization Rates in Acetate Buffer with Powdered Enamel

By W. I. HIGUCHI, P. R. PATEL, and J. J. HEFFERREN*

The initial demineralization rates of several synthetic hydroxyapatite samples and of powdered enamel in acetate buffers over a wide range of pH and buffer concentrations have been investigated. The results have been compared with the previously derived theory. Both the synthetic mineral results and the powdered enamel data compare well with theory. These findings provide considerable support to the idea that simultaneous theoretical studies and experimental studies with enamel and with synthetic minerals will lead to a clear understanding of many enamel reaction mechanisms.

THAT ACID decalcification is the first step in tooth decay was postulated years ago (1). Since then many investigators have studied the reactions of tooth mineral in acidic media (2), often for the purpose of devising tests for anti-decay agents whose actions were expected to depend on their ability to reduce enamel solubility (or the demineralization rate).

Until recently, no systematic quantitative theory for the acid demineralization of enamel existed. Then, in 1962, Gray (3) reported on some carefully conducted experiments with block enamel in a number of acids and established that a solution diffusion controlled mechanism was indeed operating. Gray was able to show that his data fitted a general empirical relationship involving the hydrogen ion concentration and the buffer acid concentration. Recently, Higuchi *et al.* (4) applied the mathematics of simultaneous diffusion and chemical reaction to several realistic models. They found remarkably good quantitative agreement between Gray's data (3) on initial enamel dissolution rates in acid buffers and the model in which it was assumed that hydroxyapatite was the thermodynamically governing phase in enamel. These significant findings have led to a research program in which the initial aims are to correlate the theory simultaneously with experiments on enamel and with experiments on synthetic minerals.

In the present paper, the results of studies with synthetic hydroxyapatite and powdered enamel are presented. The initial demineralization rate

data have been compared with theory (4). The three-way comparison is satisfactory, supporting the previous findings that the hydroxyapatite model appears to describe the data adequately.

EXPERIMENTAL

Synthetic Hydroxyapatite Samples.—Samples of hydroxyapatite from a number of different sources were selected for these studies for a number of reasons. First, the solubility behavior of hydroxyapatite has not been characterized adequately.¹ Thus, the reported values for the solubility product varies from 1×10^{-110} to 1×10^{-120} . Second, a number of investigators (6, 7) have employed commercial hydroxyapatite samples for studies designed to seek out the basic enamel mineral reactions under different conditions. Thus, it is desirable to have some indication of the variability among samples from different sources or from different methods of preparation with regard to demineralization rates.

Let us designate the five samples of synthetic hydroxyapatite as *A*, *B*, *C*, *D*, and *E*. Samples *A* and *B* were commercial samples (obtained from the Victor Chemical Works, Chicago, Ill., and the Monsanto Chemical Co., St. Louis, Mo., respectively). The other samples, *C*, *D*, and *E*, were prepared in the following manner.

Basically, the procedure of Egan *et al.* (8) was followed in the preparation of the sample designated *D*. One liter of 0.20 *M* calcium nitrate and 1 L. of 0.12 *M* monobasic ammonium phosphate were added simultaneously at the rate of 100 ml. of each per hour, with continuous stirring into a 5-gal. Pyrex carboy containing 12 L. of distilled water placed on a steam bath. The pH of the solution was maintained at 4.5 or above by adding 1.0 *M* ammonium hydroxide from a 500-ml. portion. Finally, when the addition of all of the calcium nitrate and the monobasic ammonium phosphate solutions was completed, the remainder of the ammonia solution was added dropwise to the mixture. The precipi-

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¹ This is presumably due (a) to the large specific surface areas (5) associated with most conveniently prepared samples leading to nonstoichiometric surface ionic ratios under different solution conditions and (b) to the tendency of foreign ions to substitute into the apatite lattice.

TABLE I.—COMPOSITION OF SAMPLES

Sample	% HAP by Phosphate Analysis	% HAP by Calcium Analysis
A	95.0	92.2
B	96.5	90.1
C	96.0	92.0
D and E*	99.5	100.0
Enamel powder	94.8	87.4

* Granules of sample D sintered at 900°C. for 72 hr.

tate was stirred for another 12 hr., then allowed to settle. It then was washed by decantation with distilled water, filtered, and dried at 105° for 72 hr.

The preparation of sample C differed from the above because instead of following the slow precipitation procedure, 500 ml. each of 0.40 M calcium nitrate and 0.24 M monobasic ammonium phosphate were added together during a 30-min. period to a 4-L. flask containing 1 L. of distilled water and 30 ml. of 0.1 M ammonia maintained at 90 to 95°. The resulting precipitate was digested for 1 hr. at the same temperature, then allowed to cool. Then it was filtered, washed, and dried as described for sample D.

Sample E was the portion of sample D that was sintered at 900° for 72 hr. after the granulation step described below.

The analytical data for these samples are given in Table I in terms of per cent hydroxyapatite calculated by assuming that calcium or phosphate is present as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ only. These were obtained by the analytical methods described later. The values represent the average of several determinations. The precision of the results in these instances was about $\pm 1\%$ for both the calcium and phosphate analyses.

All five samples gave the characteristic diffraction peaks for hydroxyapatite. These determinations were made on the Siemens Crystalloflex IV X-ray diffractometer.

Powdered Tooth Enamel.—The procedure for efficient grinding of tooth enamel was described recently (9). Extracted human teeth were cleaned to remove roots, decayed portions, and pulp. These were ground (9) in a model M Fitz Mill (W. J. Fitzpatrick Co., Chicago, Ill.) using a No. 60 mesh sizing screen. Following the grinding step, the powder obtained was screened, and the fraction collected between 100 and 200 mesh screen sizes was retained. Then the enamel was separated from the dentin by the floatational procedure, described by Manly and Hodge (10), using bromoform as the suspending medium. The separated particles then were given several rinses with acetone, dried overnight in air at room temperature, and finally dried in an oven for 1 hr. at 105°C.

Procedure for the Synthetic Hydroxyapatite Dissolution Experiments.—Tablets of synthetic hydroxyapatite, 0.5 in. in diameter, were made using the Carver laboratory press at 75,000 p.s.i. The tablets then were ground lightly in a glass mortar and sieved dry, without vibration, through the No. 80 and 100 mesh screens. The portion retained by the No. 100 mesh screen was used in the experiments.

A portion of sample D in this granule size range was placed in a furnace at 900° for 72 hr. of sintering. This sintered portion was designated sample E.

Exactly 100 mg. of these granules was transferred into a 250-ml. volumetric flask clamped to the arm of a Burrell Wrist-Action shaker so that the lower portion of the flask was immersed in a water bath maintained at $30.0 \pm 0.1^\circ$. Then 200 ml. of acetate buffer at $30.0 \pm 0.1^\circ$ was introduced into the flask, and the shaker was turned on. The shaker amplitude was adjusted to keep the granule particles just suspended in the medium. These agitation conditions were maintained constant for all runs. A timer was initiated with the starting of the shaker. Five-milliliter samples were withdrawn at predetermined times with a syringe-filter assembly. The syringe-filter assembly consisted of a glass filter stick (maximum pore size of 4–8 μ) connected by means of a piece of Tygon tubing to the inlet of a B.D. automatic valve attached to a 50-ml. syringe. The outlet of the valve was connected to a piece of latex rubber tubing which dipped back into the solution. This arrangement permitted sampling and then rinsing out the line before the next sample was taken. All samples were collected in premarked screw-capped tubes.

Procedure for Enamel Experiments.—Exactly 100 mg. of the enamel powder was run in a manner the same as the granulated synthetic hydroxyapatite samples.

Analysis of Calcium and Phosphate.—Calcium was analyzed according to the method of West and Lancina (11). The procedure involved measuring the absorbance of a complex formed between calcium ions and the reagent, Calcichrome,² at $\lambda = 510 \text{ m}\mu$ and $\text{pH} = 12.0$. Because phosphate interfered to some extent, the procedure was modified by adjusting the phosphate concentrations to a constant level in both the standards and the unknown solutions.

Phosphate concentrations were determined by the method of Gee *et al.* (12) in which the phosphoammonium molybdate complex formed was reduced by stannous chloride. The absorbance of the resulting blue color was determined at the end of 15 min. at $\lambda = 720 \text{ m}\mu$.

On the routine basis, the phosphate analysis yielded a precision of about $\pm 1\%$. The fluctuations in the calcium determinations were greater, averaging about $\pm 3\%$.

RESULTS

The results of the dissolution rate studies in acetate buffer are presented in Figs. 1 to 4. Here the amounts of hydroxyapatite in solution were calculated on the basis of the phosphate analysis assuming a stoichiometric reaction. The calcium analyses were performed for many of the runs with samples A, C, D, and with the enamel powder. Where both analyses were carried out, the rates calculated on the basis of either analyses gave essentially the same *initial* rates. However, for the synthetic hydroxyapatite samples, the amounts of calcium released at long times were always less than the stoichiometric amount based on the phosphate analyses. In the present paper, we are concerned primarily with the initial rates.

In Fig. 1, the results with the three samples, A, B, and C, are presented. As the rates for these

² Obtained from Burdick Jackson Laboratories, Muskegon, Mich.

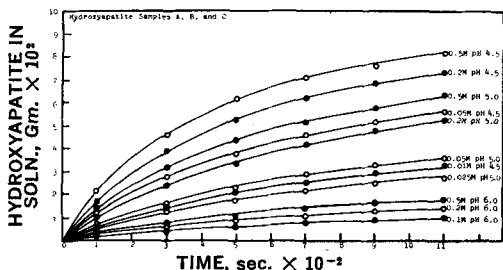


Fig. 1.—Plots showing data on dissolution of hydroxyapatite in acetate buffer with time. Samples A, B, and C gave essentially identical results in the time range given.

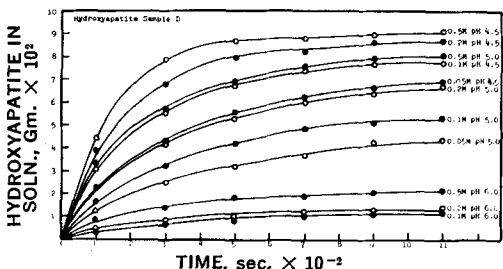


Fig. 2.—Data for sample D showing dissolution of hydroxyapatite in acetate buffer with time.

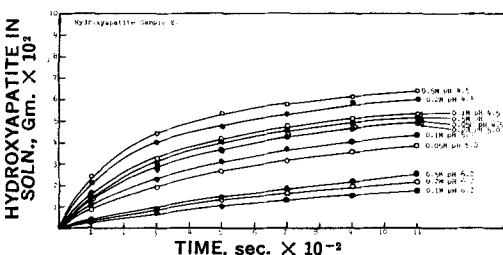


Fig. 3.—Data for sample E (sintered sample D) on hydroxyapatite dissolution in acetate buffers.

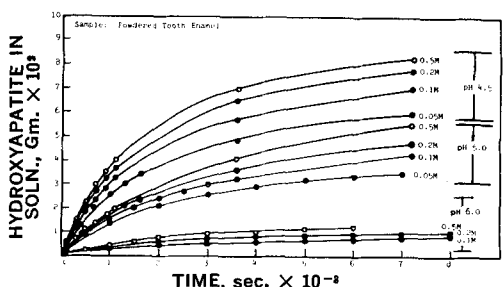


Fig. 4.—Plots showing data on dissolution of enamel powder in acetate buffer with time.

three samples were essentially identical for initial rates, only one set of data is presented. Both samples A and C were run at every pH and buffer concentration indicated in Fig. 1, and sample B was run in about one-half of the situations. While the amounts dissolved for relatively early times were nearly identical for all three samples, significant differences appeared to correlate with the appearance of the nonstoichiometry phenomena (above) whenever both were noted.

The results of similar runs with samples D and E

are shown in Figs. 2 and 3, respectively. Note that the rates with sample D were about a factor of two greater than those for samples A, B, and C and that the sintered sample, E, dissolved at about one-half of the rate for the unsintered sample, D.

In Fig. 4, the results with the enamel powder are given. These were obtained in duplicate; the precision of these results, as in the experiments with the synthetic mineral, was very good. Comparison of these enamel data with those given in Figs. 1-3 show that the enamel powder dissolved more slowly than any of the synthetic samples.

COMPARISON OF THE RESULTS WITH THEORY

In a recent study (4) equations were derived for the demineralization rate of enamel. These were based on the assumption that hydroxyapatite was the governing mineral phase. The appropriate form of these equations for initial rates in acetate buffers containing no common or otherwise reactive molecules or ions may be written (4)

$$\frac{0.6 Gh}{D_{H_2P} + \frac{D_{HP}K_{2P}}{(H^+)_o}} = \frac{D_H}{D_{H_2P}} (H^+)_h - \frac{D_H}{D_{H_2P}} (H^+)_o + \frac{D_{HB}}{D_{H_2P}} (HB)_h - \frac{D_{HB}}{D_{H_2P}} \left[\frac{D_{HB}(HB)_h + D_B(B^-)_h}{D_{HB} + \frac{D_B K_B}{(H^+)_o}} \right] - \frac{0.8 Gh}{D_{H_2P}}$$

and

$$(H^+)_o^{14} = \frac{K_2^6 PK_w^2}{K_{HAP}} \left(\frac{Gh}{D_{co}} \right)^{10} \left(\frac{0.6 Gh}{D_{H_2P} + \frac{D_{HP}}{(H^+)_o}} \right)^6$$

Here G is the stoichiometric dissolution rate, h is a hydrodynamic factor constant for constant agitation conditions, the D's are the diffusion coefficients for the species indicated by the subscripts, and the quantities in the parentheses are the concentrations of the indicated species at X = 0 or X = h, the boundaries of the diffusion layer. K_{2P}, K_{HP}, K_w, K_{HAP}, and K_B are, respectively, the second and third dissociation constants for phosphoric acid, the ion product for water, the solubility product for hydroxyapatite, and the dissociation constant for the buffer (acetic) acid. The symbols, HB, B⁻, HP⁻, and H₂P⁻, represent acetic acid, the acetate ion, the monohydrogen phosphate ion, and the dihydrogen phosphate ion, respectively.

The present experimental results may be compared with the theoretical equations if suitable values for the constants may be selected. Table II gives a set of such constants obtained by estimating activity coefficient corrections (4) and applying them to the thermodynamic constants.

TABLE II.—CONCENTRATION EQUILIBRIUM CONSTANTS ESTIMATED FOR IONIC STRENGTH = 0.1 TO 0.2

K_{2P}	2.0×10^{-7} moles L. ⁻¹
$\frac{K_2^6 PK_w^2}{K_{HAP}}$	1.6×10^{11} moles L. ⁻¹
K_B	2.2×10^{-5} moles L. ⁻¹

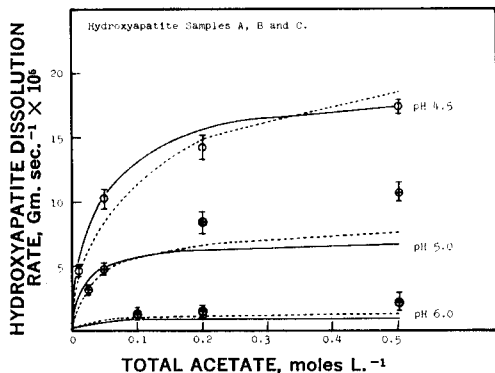


Fig. 5.—Comparison of theoretical rates with data for samples *A*, *B*, and *C*. Key: O, data; —, theory with a K_{sp} value for hydroxyapatite of 1×10^{-120} and $h = 7.9 \mu$; - - -, theory with $K_{sp} = 1 \times 10^{-113}$ and $h = 18.2 \mu$.

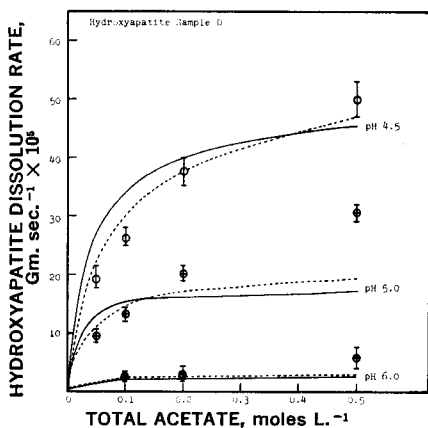


Fig. 6.—Comparison of theoretical rates with data for sample *D*. Key: same as in Fig. 5, but $h = 3.1 \mu$ and $h = 7.1 \mu$ for $K_{sp} = 1 \times 10^{-120}$ and $K_{sp} = 1 \times 10^{-113}$, respectively.

Except for K_B , these values incorporated into the theoretical equations appeared to explain satisfactorily Gray's data on enamel demineralization in lactate buffers. Therefore, their applicability in the present situation is of considerable interest.

The diffusion coefficients were all taken equal to $1 \times 10^{-5} \text{ cm.}^2 \text{ sec.}^{-1}$. This is expected to be a reasonably good assumption (4) because the diffusion of the H^+ is not expected to be important in the present range of conditions. Also, the actual value for the diffusion coefficient is unimportant in the present situation, because for relative purposes the diffusion coefficients may be absorbed into the h factor as long as they are all set equal to each other.

In Figs. 5-8, the initial rates of dissolution determined from the curves in Figs. 1-4 are plotted as a function of total buffer concentration at the three pH values. These rates were estimated two ways, and the uncertainties indicated in the plots represent these variations rather than the experimental precision, which was better. The first method of estimating initial rates involved the manual extrapolation of the data points in Figs. 1-4 to the abscissa and estimating the initial slopes. The second procedure involved an estimation of the slopes at the point where 10% (10 mg.) of the sample had dis-

solved and a correction of these values for the reduction in driving force for dissolution estimated from the amount dissolved at that time and the amount in solution at infinite time, *i.e.*, the solubility.

The closed curves in Figs. 5-8 represent theory based on the equations and the values given in Table II. For each set of data (all three pH values and all buffer concentrations) a single h value was selected to give the best visual fit, as shown in each of the figures. The broken curves in Figs. 5-8 correspond to theory based on a value of

$$\frac{K_{sp}^3 K_w^2}{K_{HAP}} = 1.6 \times 10^4$$

instead of the one given in Table II. This corresponds to a K_{HAP} value of 1×10^{-113} , instead of 1×10^{-120} employed in calculating the Table II value.

In general, the comparisons of both the enamel powder rates and the synthetic mineral rates with

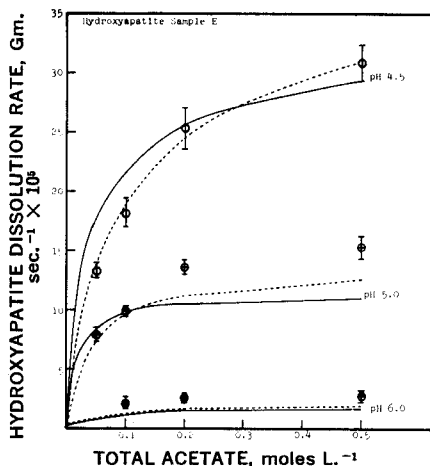


Fig. 7.—Comparison of theory with data for sample *E* (sintered sample *D*). Key: same as in Fig. 5, but $h = 5.0 \mu$ and $h = 11.0 \mu$ for $K_{sp} = 1 \times 10^{-120}$ and $K_{sp} = 1 \times 10^{-113}$, respectively.

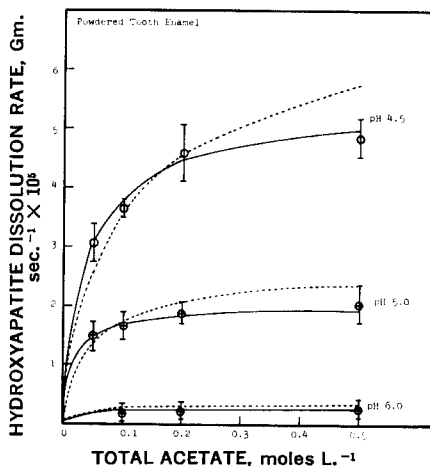


Fig. 8.—Comparison of theory with enamel data. Key: same as before, but $h = 43 \mu$ and $h = 90 \mu$ for $K_{sp} = 1 \times 10^{-120}$ and $K_{sp} = 1 \times 10^{-113}$, respectively.

theory, employing reasonable values for the parameters, are good when relative rates are considered. In the case of the synthetic mineral rates, the relative pH and buffer concentration dependences of the *D* and *E* samples are similar to those for the *A*, *B*, and *C* samples, despite differences in absolute rates. The use of $K_{\text{HAP}} = 1 \times 10^{-11.3}$ appears to give somewhat better agreement³ of the synthetic mineral data with theory, but the reverse is true in the case of enamel powder. The enamel data agree remarkably well with the theory employing those same constants that gave good agreement between theory and Gray's data (3).

Continuing comparative studies include the effects of ionic strength, the effects of common ions and foreign reactive ions, such as fluoride, and a more detailed examination of the dissolution behavior near saturation. It is believed that these

³ It is noteworthy that recent (13) solubility studies with well characterized hydroxyapatite samples indicate that the K_{sp} may be of this order of magnitude or even greater.

investigations will lead to a clear understanding of the mechanisms involved in the acid attack of enamel and in enamel reactions in general.

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Percutaneous Corticosteroid Absorption Correlated to Partition Coefficient

By M. KATZ and Z. I. SHAIKH

The vasoconstriction produced in human skin by topical corticosteroids was utilized by McKenzie and Stoughton as a biological method for measuring the efficiency of percutaneous absorption. Correlation with experimental results in this report seems to indicate that the efficiency of percutaneous absorption may be a function of physical constants, such as the product of the partition coefficient and the square root of the aqueous solubility. The results are in agreement with theoretical considerations developed by Higuchi. This suggests that increases in topical corticosteroid anti-inflammatory activity, produced by molecular modifications, are in great measure proportional to changes in solubility and partition coefficient.

IT APPEARS THAT the water-lipid partition coefficient, originally postulated in the Meyer-Overton theory, is actually important for the absorption of substances through the skin. Those substances which combine lipoid solubility with a moderate aqueous solubility are soluble in the sebum, readily penetrate into the skin, then dissolve in the tissue fluids (1).

Valette (2) found that the cutaneous penetration of hydrocarbons, alcohols, and esters was related to liposolubility and viscosity and could be closely approximated by the rate of travel on porous porcelain impregnated with fatty acids. Treherne (3) showed that the permeability of excised skin for several radiotagged substances paralleled their ether/water partition coefficients. Stoughton and Clendenning (4) found a correlation between the penetration of the epidermal barrier by a series of nicotinic acid derivatives

and their ether/water partition coefficients. They also found a similar correlation between the benzene/water partition coefficients and the penetration of the epidermis by a series of closely related boronic acid derivatives (5). The magnitude of the effect of moisture on the percutaneous diffusional rates of several salicylate esters has been shown by Wurster and Kramer (6) to be proportional to the oil-water distribution coefficient and the water solubility of these closely related compounds.

McKenzie and Stoughton (7, 8) recently have devised a technique for utilizing human skin vasoconstriction as an index of the percutaneous absorption of steroids. Since the most powerful vasoconstrictors in their series were those steroids which have been shown to be the most effective topical anti-inflammatory agents, their vasoconstriction index also might be considered an index of relative potency. The precise ranking and groupings they obtained with their *in vivo* technique suggested that the relative activity of the steroids might be correlated with *in vitro*

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