DISSOLUTION OF TEETH ENAMEL

AS A RESULT OF

ORAL MICROBIAL GROWTH

BT303

Bioreaction Engineering II

CHOOSE-FOCUS-ANALYZE (CFA) EXERCISE

BY

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Abstract

A large number of bacteria live in the oral cavities of organisms. Most of them colonize teeth surfaces and are facultative anaerobes. They ferment sugars taken by the host and produce acids which dissolve the protective surface of the teeth. Such teeth wear out faster and start decaying as newer species of bacteria prosper. The extent of damage caused by the acid is however dependent on numerous factors like oral hygiene, eating habits, time of contact, teeth and salivary composition etc. Anaerobic growth of microorganisms results in a lowered pH but salivary flow acts as a buffering solution maintaining the oral pH and providing materials for teeth reformation. This report describes the use of the Ludeking- Piret model for acid production and variation of acid concentration contacting teeth enamel and its dissolution with time. It was found that the rate of dissolution slows down initially and then increases exponentially with time. Further the solubility of enamel increases very rapidly when pH drops below 5.

Introduction

Enamel is the protective coat found on the visible portions of teeth above the gum line. It is the hardest substance in human body and is over 95% mineral in composition. The main structural component of the enamel is a mineral called hydroxyapatite which is chemically Ca_{10} $(PO_4)_6$ $(OH)_2$ and has frequent presence of carbonates and fluorides as impurities.

The function of the enamel is to protect teeth from wear and tear. The enamel coating is constantly damaged due to normal exposure to food and liquids and also as a result of the action of microbes that live on teeth. However it normally does not get depleted owing to regular remineralization processes operating in the oral cavity. There is always a balance between degradation and reformation which maintains the enamel in a healthy individual. Excessive colonization of dental surface due to lack of regular oral hygienic practices can however lead to a condition known as dental caries. In such a situation, the bacterial acid production and thus the rate of destruction of enamel coat is much faster than the natural rate of remineralization.

The oral inhabitants

Oral cavity being both a good shelter and a source of nutrition is home to a large variety of microorganisms. The human oral microflora is diverse and is usually predominately composed of Gram-positive bacteria. Oral bacteria include streptococci, lactobacilli, staphylococci and corynebacteria, with a great number of anaerobes, especially bacteroides. *Streptococcal species* ----*S. milleri, S. mutans, S. salivarius, S. mitior and S. sanguis* are almost always present in plaques and caries, the dominant species being *S. sanguis* and *S. mutans*. *Streptococcus mutans* appears to be important in the initiation of dental caries because its activities lead to colonization of the tooth surfaces, plaque formation, and localized demineralization of tooth enamel. Once enamel is weakened, other bacteria also colonize the damaged region. These include Lactobacilli, *Actinomyces,* and various proteolytic bacteria which eventually enter the interior of teeth.

Acid is produced when bacteria grow in the absence of oxygen. Such environments are formed in the pockets of teeth under bacterial films. Energy in this case is derived solely from the glycolytic process and the final product which is mostly lactic acid is secreted out of the cell. This acid reduces pH of the medium affecting the oral health of the host.

Remineralization

Remineralization is a natural process in which inorganic minerals in saliva are deposited on carious dental surfaces under appropriate conditions, restoring the mineral content of teeth. The effect of this process varies greatly among individuals depending upon enamel composition, oral health and salivary constituents. An equilibrium always exists between the solvated and solid mineral as

$$Ca_{10}(PO_4)_6(OH)_2 \leftrightarrow 10Ca^{2+} + 6PO_4^{3-} + 2OH^-$$

The solubility of hydroxyapatite in water is extremely low as is evident from its solubility product $K_{sp}=10^{-117}$. Thus it is not the dissolution of enamel that weakens the teeth surface. Any compound dissolves in a solution till its ionic product is less than its K_{sp} and conversely the compound gets precipitated if its ionic product is higher than $K_{sp.}$ It has been found that the concentration of phosphate in solution decreases markedly as pH is lowered. So at lower pH, higher amounts of calcium are released from the mineral structure as both hydroxide and phosphate concentrations are low. This explains the higher rates of enamel demineralization at lower pH.

Remineralization is essentially a reversal of the conditions that cause demineralization. Minerals from food or saliva get dissolved in carbonic acid formed momentarily from the CO_2 in breath and are deposited at the damage site of the enamel structure as the acid dissociates. However this process is naturally inefficient in recalcifying acid eroded enamel surfaces as they are always covered by a pellicle of salivary and bacterial proteins. Thus remineralization helps only if the enamel layer is intact as in a healthy individual or in the white caries lesions. These lesions are formed due to decalcification of inner tooth material and can be treated by enhancing salivary flow (to increase remineralization) and maintaining good hygiene.

In this report a mathematical formulation has been proposed to describe the effects of bacterial growth on enamel. Main assumptions that have been made are as follows

a. The growth of bacteria is *not* limited by nutrient source.
   This assumption has been made keeping in view the popular eating habits and the report that *Streptococcus* bacteria can use saliva for growth.
b. Growth of the bacteria is *non-competitively* inhibited by the acid product.
c. The dilution and buffering effects of the saliva do not affect acid concentrations in the layer of fluid contacting enamel.
d. Remineralization is negligible during the period considered.
e. No factor other than product affects the growth of bacteria.

Dissolution of enamel

Considering the Ludeking-Piret model for acid production

$$r_p=\alpha r_x+\beta x \qquad \ldots(1)$$

where

α = growth dependent parameter

β = growth independent parameter

The expression for the specific growth rate using the Monod Model and considering the inhibitory affect of high product concentrations is

$$\mu = \frac{\mu_m S}{(K_s + S)} \cdot \frac{K_p}{(K_p + P)} \qquad \ldots(2)$$

where

μ_m= max. sp. growth rate

K_s, K_p are constants

The effect of substrate concentration on the specific growth rate can be neglected considering the abundance of substrate and hence assuming that $S >> K_s$. Also the product is assumed to inhibit cell growth due to a change in the pH of the medium. In such a case we have from (2)

$$\mu = \frac{\mu_m K_p}{(K_p + P)}$$

Using this in (1)

$$r_p = \frac{dP}{dt} = \alpha r_x + \beta x = \alpha \mu x + \beta x = \alpha \frac{\mu_m K_p x}{(K_p + P)} + \beta x \qquad \ldots(3)$$

Also $P = Y_{p/x} (x - xo)$

$\Rightarrow x = xo + Y_{x/p} P$

Hence by (3)

$$\frac{dP}{\left(\frac{\alpha \mu_m K_p}{(K_p + P)} + \beta \right) (xo + Y_{x/p} P)} = dt$$

$$\Rightarrow \frac{(K_p + P)\, dP}{\{\alpha \mu_m K_p + \beta (K_p + P)\} (xo + Y_{x/p} P)} = dt$$

$$\Rightarrow \int \frac{(K_p + P)\, dP}{\{\alpha \mu_m K_p + \beta (K_p + P)\} (xo + Y_{x/p} P)} = t$$

Defining $A = \left(\frac{\alpha \mu_m + \beta}{\beta} \right) K_p$ and $B = \frac{xo}{Y_{x/p}}$

On integration we get

$$t = \frac{1}{\beta Y_{x/p} (A - B)} \ln \left[\frac{(B + P)^{Kp-B}}{(A + P)^{Kp-A}} \cdot \frac{B^B}{A^A} \right] + \text{const}$$

For given xo and constants α, β, $Y_{x/p}$, μ_m and K_p, A and B are constants.

Therefore we have

$$t = \frac{1}{\beta Y_{x/p}\,(A-B)} \ln\left[\frac{(B+P)^{Kp-B}}{(A+P)^{Kp-A}}\right] + C \qquad ;\ C = \text{new const}$$

At t=0 assuming P=0

$$\Rightarrow C = \frac{-1}{\beta Y_{x/p}\,(A-B)} \ln\left[\frac{(B)^{Kp-B}}{(A)^{Kp-A}}\right]$$

Hence

$$t = \frac{1}{\beta Y_{x/p}\,(A-B)} \ln\left[\frac{(1+P/B)^{Kp-B}}{(1+P/A)^{Kp-A}}\right]$$

$$\Rightarrow t = \frac{1}{\beta Y_{x/p}\,(A-B)} \ln\left[(1+P/B)^{Kp-B}\,(1+P/A)^{A-Kp}\right] \qquad \ldots(4)$$

The acid that is produced by the fermentation process consists mainly of lactic acid with small amounts of acetate, succinate and formate due to the presence of mixed culture. So its properties can be considered to be that of lactic acid.
The dissociation constant K_a for lactic acid is 8.3×10^{-4}.
Considering the dissociation reaction to be

$$HA \Leftrightarrow H^+ + A^-$$

$$K_a = \frac{[H^+]\,[A^-]}{[HA]}$$

Assuming that the acid is weakly dissociated, the H^+ concentration in the solution is

$$[H^+] = \sqrt{(K_w + [HA] \times K_a)} = \sqrt{(K_w + P \cdot K_a)}$$

where K_w is the dissociation constant of water.

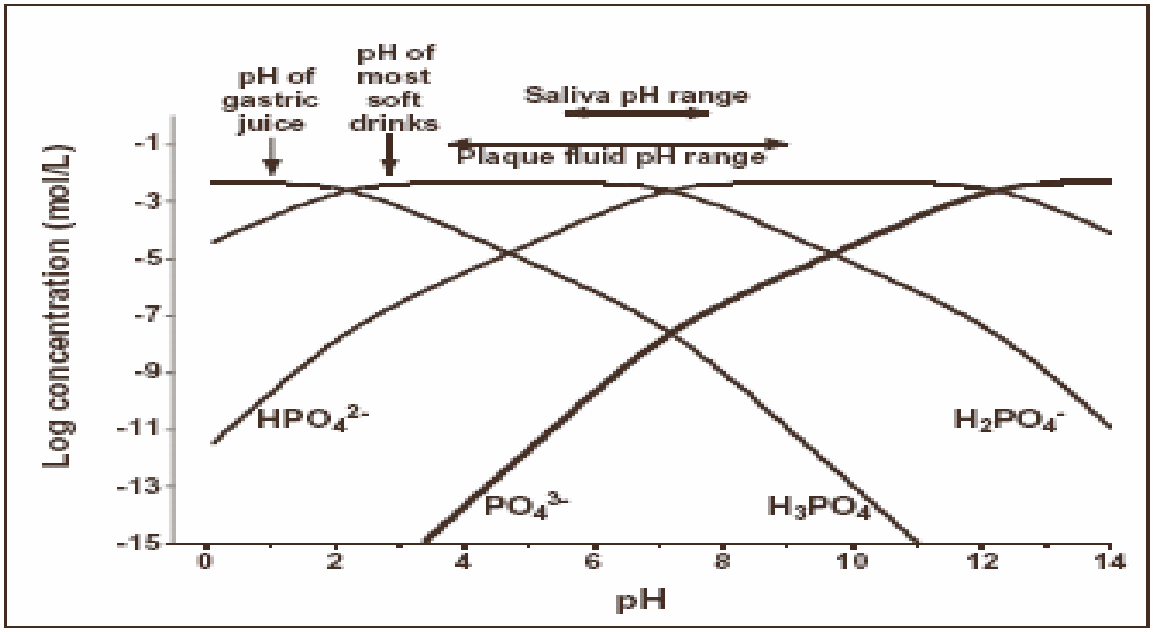
$$\therefore\ P = \frac{[H^+]^2 - K_w}{K_a} \text{ mol/l} = 90\,\frac{[H^+]^2 - K_w}{K_a} \text{ g/l} \qquad [\text{Mol wt of lactic acid} = 90]$$

Hence the relation between time and pH of the medium surrounding enamel is

$$t = \frac{1}{\beta Y_{x/p}\,(A-B)} \ln\left[\left(1+\frac{90\,[H^+]^2 - K_w}{BK_a}\right)^{Kp-B}\left(1+\frac{90\,[H^+]^2 - K_w}{AK_a}\right)^{A-Kp}\right] \qquad \ldots(5)$$

Here A,B,K_p are in g/l and H^+ concentration is in moles/l.

The variation in the concentration of phosphate at different pH [ref (2)] has been found to be as shown below:



The region of interest to us can be approximated by a straight line corresponding to variation of PO_4^{3-} in the pH range from 3 to 7.

At pH=4, Log ($[PO_4^{3-}]$) = -13.6 and slope of the line is approximately 1.95
Therefore equation of the straight line is

$$\text{Log}([PO_4^{3-}]) = -13.6 + 1.95(\text{pH} - 4) = -13.6 - 1.95(\text{Log}([H^+]) + 4)$$

$$\Rightarrow \text{Log}([PO_4^{3-}]) = -21.4 - 1.95\,\text{Log}([H^+]) \quad \ldots(6)$$

For hydroxyapatite

$$K_{sp} = 10^{-117} = [Ca^{2+}]^{10}\,[PO_4^{3-}]^6\,[OH^-]^2$$

$$\therefore [Ca^{2+}]^{10} = \frac{10^{-117}}{[PO_4^{3-}]^6\,[OH^-]^2} = \frac{10^{-117}}{[PO_4^{3-}]^6 \times \frac{K_w^2}{[H^+]^2}} = \frac{10^{-89}[H^+]^2}{[PO_4^{3-}]^6} \quad \ldots(7)$$

At any given time t, the hydronium ion concentration can be determined from equation (5) and then used in equation (6) to get phosphate concentration in solution. These can then be used in equation (7) above to get the equilibrium calcium ion concentration in the solution.
Assuming that at t=0 there were no calcium ions in the medium and hence the calculated amount was generated by dissolution of hydroxyapatite (Ha), the amount of the mineral dissolved in *unit volume* of the solution is given by

$$\text{Ha} = 0.1\,[Ca^{2+}] \quad \text{moles/l}$$

Mol wt of Ha is 1004 g/mol.
Hence mass of Ha dissolved per unit volume of solution

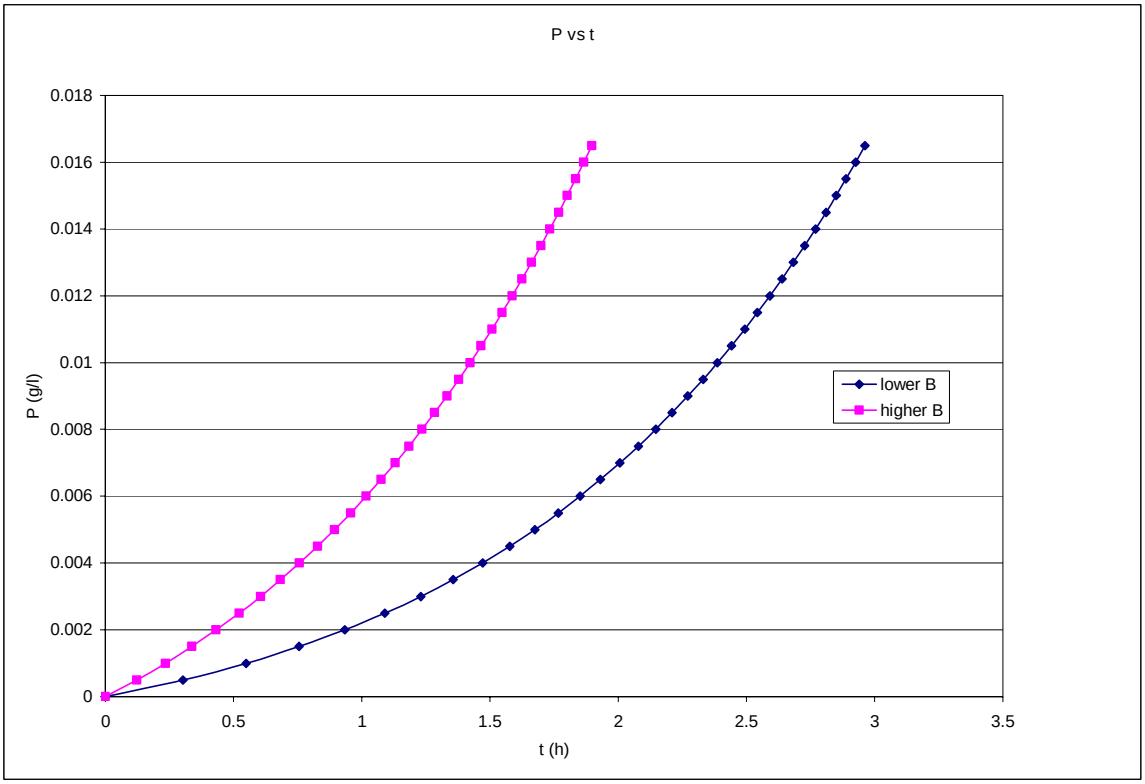
$$\text{Ha} = 100.4\,[Ca^{2+}] \quad \text{g/l.} \quad \ldots(8)$$

Results and discussion

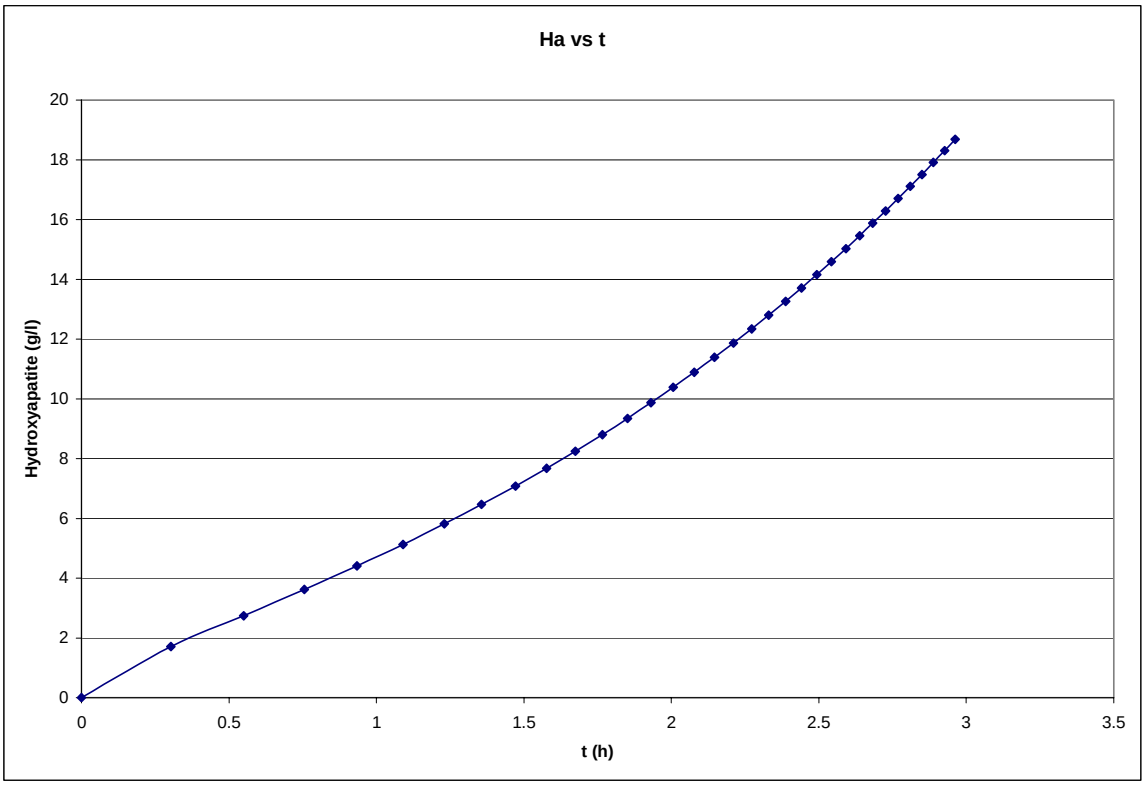
Experimental values for all the parameters used in the above formulation were not available. To get a final numerical result the following data and estimates were used:

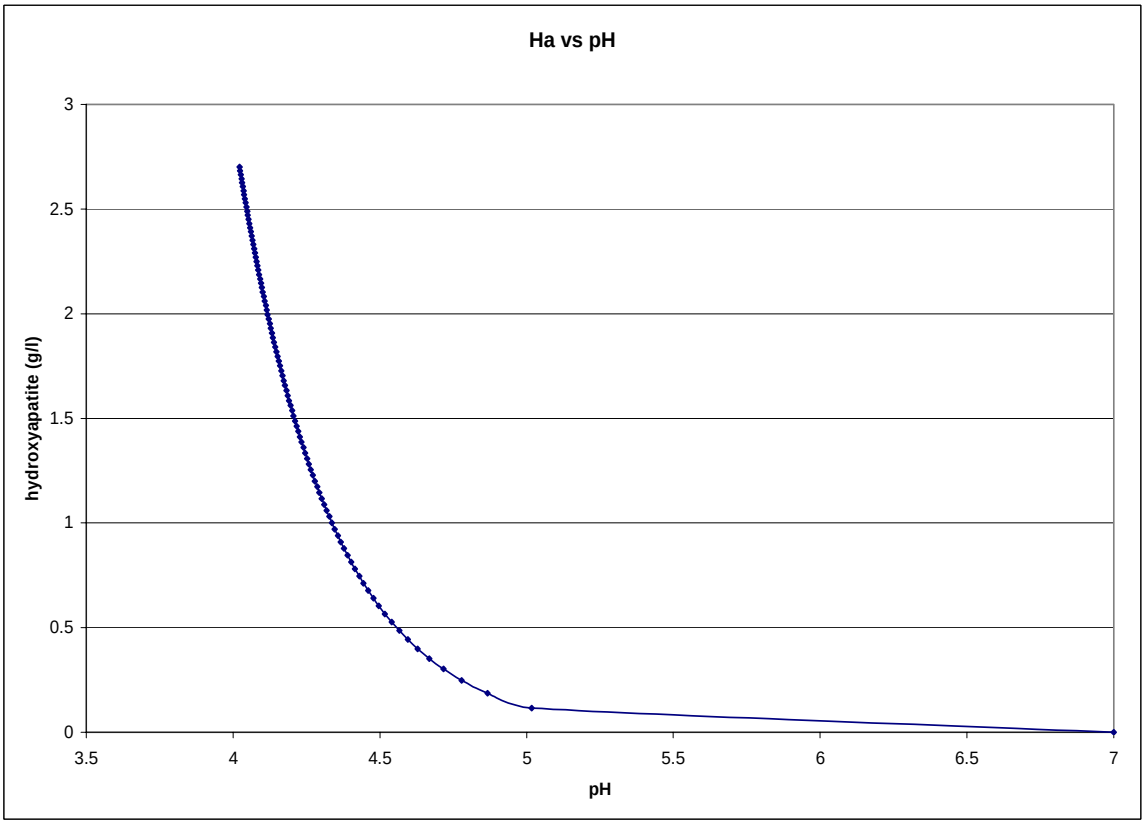
1. From ref (1) for *S. mutans* grown on saliva and glucose, the recovery of carbon calculated on the basis of fermentation products formed from the glucose added to the culture was 59%. Taking the recovery to be 0.6 and assuming carbon content of the cell to be 50%, we get
   $Y_{x/p} = \frac{(1-0.6)/0.5}{(0.6x90)/(12x3)} = 0.533$ g/g or $Y_{p/x} = 1.875$ g/g
2. Also from ref (1), the doubling time for *S. mutans* was 3 h. Therefore $\mu_m = (\ln 2)/3\ h^{-1} = 0.231\ h^{-1}$
3. Typically the bacterial count in the saliva is 10^6 cells/ml and mass of a bacterium is 10^{-12}g. As an estimate of initial concentration of bacteria in the fluid contacting enamel, I assume that $xo = 10^{-3}$g/l. Therefore $B = \frac{xo}{Y_{x/p}} = 1.875 \times 10^{-3}$g/l
4. From ref (4) product inhibition constant for S. mutans, $K_p = 4.24$ mM = 0.38 g/l
5. Values of the parameters α and β could not be found for streptococcus bacteria. As an estimate, the values $\alpha = 5$ g/g and $\beta = 0.3$ g/g/h have been used.(the values are typical of lactococcus)

Using these estimates, $A = (\alpha \mu_m/\beta + 1) K_p = 20.56$ mM $= 20.56 \times 90$ mg/l $= 1.85$ g/l
The variation of acid concentration (P) and hydroxyapatite concentration (Ha) were plotted with time (t).The results are shown below:



Higher B=2.5 times lower B


Ha vs t
Hydroxyapatite (g/l)
t (h)
0
2
4
6
8
10
12
14
16
18
20
0
0.5
1
1.5
2
2.5
3
3.5


Ha vs pH
hydroxyapatite (g/l)
pH
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7

Analysis of Ha vs. t plot shows that the rate of dissolution as given by the change in the concentration of Ha with time is quite high initially (found to be about 6.4 g/l/h). Thereafter it slows down reaching a minimum of 4.2 g/l/h at 0.7 h and then increases continuously with time overtaking the initial rate at about 1.8 hours. After this time, the dissolution is much more rapid and is mainly responsible for the loss of enamel.
Plot of Ha vs. pH shows the solubility as a function of pH. Enamel is negligibly soluble at neutral pH. The trend in the curve shows that enamel solubility increases rapidly below the pH of 5. Normal pH of saliva in humans varies between 5.5 and 6.5 in which the solubility is quite low and hence saliva acts as a protective solution of enamel. Needless to say, activities that enhance salivary flow (like chewing gum) are effective in protecting teeth from the acid damage. Another approach to teeth care is to use fluoridated toothpastes or traces of fluoride in drinking water, which causes a reduction in the solubility of enamel. Fluoride replaces the hydroxyl groups in the hydroxyapatite structure and hence prevents rapid hydrolysis of the mineral. Timing of snacks has also been found to be an effective way of controlling tooth decay.
The effect of fruit juices and soft drinks on teeth can be estimated by their pH. The pH of most soft drinks lies around 3 suggesting that teeth surface exposed to these drinks can dissolve very rapidly. However natural coating of salivary proteins and polysaccharides on the teeth reduces the effect of such activities. The major threat is still the film of bacteria adhering to the enamel and its time dependent acid production. As can be seen from the plot of acid concentration vs. t for different B (and effectively different initial microbial concentration) oral hygiene can play a major role in determining the susceptibility of teeth to decay. Regular and proper brushing of teeth will reduce xo and the time available for the pH of the medium surrounding the enamel to increase to cariogenic levels.

Conclusion

It has been theoretically found that enamel dissolution rates are not constant and vary with time and initial conditions. Some interesting patterns in the variation of enamel solubility with pH and time have been found but it remains to be seen whether they are inherent in the model assumptions or are actually observed for the dissolution process. The actual process of enamel dissolution is quite complex and many factors such as buffering action of saliva, diffusive transport of materials, side products of bacterial growth, remineralization and microenvironments are expected to play critical roles. Hence the model discussed in this report is overly simplified and so is least expected to cover all the aspects of the topic. Some assumptions can limit its applicability to general case and so it is not very reflective of the actual dissolution process. Nonetheless it is expected to represent some salient features of the processes involved in the action of oral bacteria on teeth enamel.

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