Enamel dissolution in citric acid as a function of calcium and phosphate concentrations and degree of saturation with respect to hydroxyapatite

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The aim of this study was to investigate enamel dissolution in citric acid solutions as a function of solution calcium and phosphate concentrations and degree of saturation with respect to hydroxyapatite (DS_{HA}). The primary relevance of the study is the development of soft drinks with reduced erosive potential. Nanoindentation was used to investigate changes in the hardness of polished human enamel surfaces after 120 s and 300 s exposure to solutions with pH 3.30 and a range of calcium and phosphate concentrations. All solutions were undersaturated with respect to hydroxyapatite, with $0.000 \leq DS_{HA} \leq 0.295$. A complex dependence of enamel softening on calcium concentration was observed. Substantial enamel softening occurred in solutions with calcium concentrations equal to or less than 120 mM ($DS_{HA} \approx 0.104$), but there was little or no statistically significant softening of the enamel for calcium concentrations over 120 mM. This condition may be applicable to soft drink formulation. Furthermore, solutions with $DS_{HA} = 0.101$ and different calcium/phosphate ratios resulted in different degrees of softening of the enamel. Hence, contrary to assumptions made in many models, enamel dissolution is not simply a function of DS_{HA}, and individual calcium and phosphate concentrations are critical.

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The dissolution of dental enamel in acidic solution is a function of several factors, including calcium and phosphate concentration, degree of saturation with respect to hydroxyapatite (DS_{HA}), pH, and acid concentration and type (1–3). The DS_{HA} is determined by the concentrations of calcium and phosphate in the solution and by the pH (4). Several studies have shown that the addition of calcium and/or phosphate to an acidic solution has a pronounced effect on enamel dissolution (3, 5–7). These studies, however, addressed a narrow range and small number of calcium and phosphate concentrations, and many used prolonged exposure times. The dependence of enamel dissolution on calcium and phosphate concentrations and DS_{HA} over a wide range of values is therefore not known. It is particularly important to investigate this dependence over physiologically relevant time-scales of a few minutes, which are comparable to clearance times of acids in the mouth (8).

The relationship $R \propto (1 - DS_{HA})^d$, where R is the rate of enamel or hydroxyapatite dissolution and d is the order of reaction associated with DS_{HA}, has been discussed many times (3, 6, 9–12). Implicit in the consideration of this relationship is the assumption that DS_{HA} is the important parameter in determining the rate of enamel dissolution and that the individual calcium and phosphate concentrations are not important. Most authors, however, do not discuss or test this assumption.

The relationship between enamel dissolution and calcium and phosphate concentration is of importance to the formulation of soft drinks (13). Soft drinks are known to cause dental erosion *in vitro* and *in situ* (14). Typical soft drinks have pH $\approx$ 3.30 (15), a calcium concentration of $\approx$ 1 mM (16) and a phosphate concentration of $\approx$ 2.4 mM (17), resulting in $DS_{HA} \approx 0.02$. Several methods of reducing the erosive potential of soft drinks are known, the most common methods being increasing the pH and the adding calcium- and/or phosphate-containing salts (13). For example, a soft drink with reduced erosive potential that has been discussed in the literature has an elevated pH of 3.8, a calcium concentration of 10 mM and a phosphate concentration of 0.5 mM, giving a $DS_{HA} = 0.11$ (18). However, these approaches can be associated with a less acceptable drink taste (13), and a higher pH results in a formulation with increased susceptibility to

microbiological spoilage (bacterial growth is inhibited at low pH). It is desirable to improve the formulation of soft drinks with reduced erosive potential, to give a good taste and a satisfactory shelf life.

Nanoindentation has recently been applied to the investigation of enamel dissolution (19, 20). It has been shown to be very sensitive to the initial stages of dissolution both *in situ* (19) and *in vitro* (20, 21). Atomic force microscopy (AFM) has been shown to discriminate between very slow rates of dissolution by different soft drinks *in vitro* (18). The study presented here was performed to investigate the reduction in erosive potential of a model soft drink with typical drink pH (15) by the addition of calcium and phosphate. The initial stages of enamel dissolution were investigated by measuring enamel hardness using nanoindentation and observing changes in surface morphology using AFM, with exposure times typical of citric acid clearance times *in vivo* (8). The study was composed of two parts. First, the hardness and morphology of enamel surfaces were compared after exposure to citric acid solutions with a wide range of calcium concentrations and DS_{HA} and a constant phosphate concentration. Second, the hardness of enamel surfaces was compared after exposure to citric acid solutions with the same DS_{HA} but different calcium to phosphate ratios, to test the hypothesis that individual calcium and phosphate concentrations are not important in determining enamel dissolution rate.

Material and methods

Erupted human molars were stored in tap water with thymol after removal of the roots and pulp. The teeth were carefully examined for evidence of caries, and the enamel and dentine surrounding any such areas were removed prior to sample preparation. Enamel sections ($n = 232$) were cut from sound enamel from the buccal and lingual sides of 60 teeth using a diamond saw. The sections were embedded in epoxy resin (Stycast; Hitek Electronic Materials, Scunthorpe, UK) and polished parallel to the natural surface using 1200 grit silicon carbide paper and 0.25 μm aluminum oxide powder. This resulted in an exposed area of enamel of approximately 2–5 mm^2. Samples were cleaned by ultrasonication in ethanol for 2–3 min before and after polishing.

The enamel samples were divided into three groups. Group 1 comprised 120 samples taken from 39 teeth, group 2 comprised 72 samples from 20 teeth, and group 3 comprised 40 samples from 28 teeth. The compositions of the solutions used for groups 1, 2, and 3 are shown in Table 1. In this paper, the notations [Ca] and [PO$_4$] are used to represent calcium and phosphate concentrations, respectively. Eight samples in each group were randomly assigned to one of the solutions using random numbers generated using atmospheric noise (http://www.random.org). Solutions were prepared using citric acid, CaCO$_3$ and H$_3$PO$_4$ (Sigma-Aldrich, Poole, UK) and distilled water. The pH of the solutions was measured using a pH meter (HI 9321 Microprocessor pH meter; Hanna Instruments, Leighton Buzzard, UK) and was adjusted to pH 3.30 by addition of HCl or NaOH as applicable. Although this resulted in

Table 1

Compositions of the solutions investigated using sample groups 1, 2, and 3 of this study

Sample group	Citric acid (mM)	Calcium (mM)	Phosphate (mM)	DS_{HA}	Exposure time (s)	Factors investigated
1	19.1	0	0	0.000	120	[Ca], DS_{HA}, t
1	19.1	2	0.57	0.016	120	[Ca], DS_{HA}, t
1	19.1	4	0.57	0.023	120	[Ca], DS_{HA}, t
1	19.1	8	0.57	0.032	120	[Ca], DS_{HA}, t
1	19.1	16	0.57	0.045	120	[Ca], DS_{HA}, t
1	19.1	24	0.57	0.053	120	[Ca], DS_{HA}, t
1	19.1	40	0.57	0.065	120	[Ca], DS_{HA}, t
1	19.1	60	0.57	0.077*	120	[Ca], DS_{HA}, t
1	19.1	80	0.57	0.086*	120	[Ca], DS_{HA}, t
1	19.1	120	0.57	0.104*	120	[Ca], DS_{HA}, t
1	19.1	160	0.57	0.121*	120	[Ca], DS_{HA}, t
1	19.1	240	0.57	0.158*	120	[Ca], DS_{HA}, t
1	19.1	360	0.57	0.220*	120	[Ca], DS_{HA}, t
1	19.1	480	0.57	0.295*	120	[Ca], DS_{HA}, t
2	19.1	0	0	0.000	300	[Ca], DS_{HA}, t
2	19.1	8	0.57	0.033	300	[Ca], DS_{HA}, t
2	19.1	24	0.57	0.053	300	[Ca], DS_{HA}, t
2	19.1	60	0.57	0.077*	300	[Ca], DS_{HA}, t
2	19.1	120	0.57	0.104*	300	[Ca], DS_{HA}, t
2	19.1	240	0.57	0.158*	300	[Ca], DS_{HA}, t
2	19.1	360	0.57	0.220*	300	[Ca], DS_{HA}, t
2	19.1	480	0.57	0.295*	300	[Ca], DS_{HA}, t
3	19.1	0	0	0.000	120	[Ca]/[PO$_4$], DS_{HA}
3	19.1	30	3.0	0.101	120	[Ca]/[PO$_4$], DS_{HA}
3	19.1	12.0	10.2	0.103	120	[Ca]/[PO$_4$], DS_{HA}
3	19.1	5.5	55	0.100	120	[Ca]/[PO$_4$], DS_{HA}

DS_{HA}, degrees of saturation with respect to hydroxyapatite; values marked * are approximate because of the high ionic strength of these solutions. The factors investigated by each sample group are indicated, and include calcium concentration, [Ca], DS_{HA}, exposure time, t, and calcium phosphate ratio. [Ca]/[PO$_4$].

relatively high concentrations of chloride in some solutions, the Cl⁻ ion and the resultant high ionic strength have a small effect on DS_{HA}, and are taken into account in the calculation. The DS_{HA} values of the solutions were calculated using a computer program based on a modified form of the Debye–Hückel theory (4). The calculation only provides approximate DS_{HA} values for solutions with an ionic strength (IS) > 0.1. These approximate DS_{HA} values are indicated by asterisks in Table 1.

Group 1 and 3 samples were exposed to 50 ml of the appropriate solution, stirred using a magnetic stirrer operated at 500 r.p.m., for 120 s. Group 2 samples were exposed in the same manner for 300 s. Each enamel sample was exposed to the appropriate solution individually, and each 50 ml batch of solution was used only once. After exposure, the samples were rinsed in gently running distilled water for 10 s. Excess liquid was removed from the enamel by touching absorbent paper to the edge of the resin, and the enamel surface was allowed to dry fully in air before analysis. The study was performed at room temperature (23.6 ± 0.1°C). One set of samples in each group was not exposed to any solution and was used as a control.

Nanoindentation was used in air to measure the hardness of the enamel samples using a Hysitron Triboscope nanoindenter (Hysitron, Minneapolis, MN, USA) on a Nanoscope IIIa atomic force microscope (Digital Instruments, Santa Barbara, CA, USA). The enamel surface was imaged using the nanoindenter tip before each indentation to ensure that the surface was clean, locally flat, and free from damage. Each sample was indented five times in different areas. Hardness was calculated by the Hysitron software using the method of OLIVER & PHARR (22). The atomic force microscope was also used in tapping mode to obtain images of enamel samples to characterize the surface morphology.

For each sample, the five values of hardness obtained were not independent as they were obtained from a single sample, and the mean value was therefore taken as a single value for each sample. The data were found not to be normally distributed, therefore a Kruskal–Wallis test and box-whisker plots were used to identify statistically significant differences within the data at a 95% confidence level.

Results

Groups 1 and 2 samples

There was a statistically significant difference between the hardness of samples exposed to different solutions and that of the untreated samples in group 1 (P < 0.00001) and group 2 (P < 0.00001). The median hardness of the group 1 and 2 enamel samples as a function of solution calcium concentration is shown in Figs 1 and 2, respectively. Each data point represents a total of 40 hardness measurements on eight enamel samples, and the error bars represent 95% confidence intervals.

It can be seen in Figs 1 and 2 that, in terms of hardness, there was a very pronounced dependence of enamel dissolution on calcium concentration at a [Ca] < 120 mM. In this range, a small increase in calcium concentration caused a large increase in enamel hardness. This was true for both group 1 and 2 samples; that is, samples exposed for 120 s and 300 s. For a
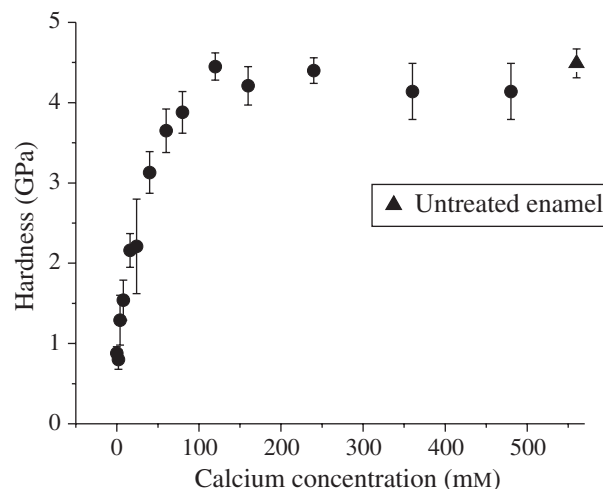

Fig. 1. Hardness of group 1 enamel samples as a function of citric acid solution calcium concentration. Samples were exposed for 120 s.
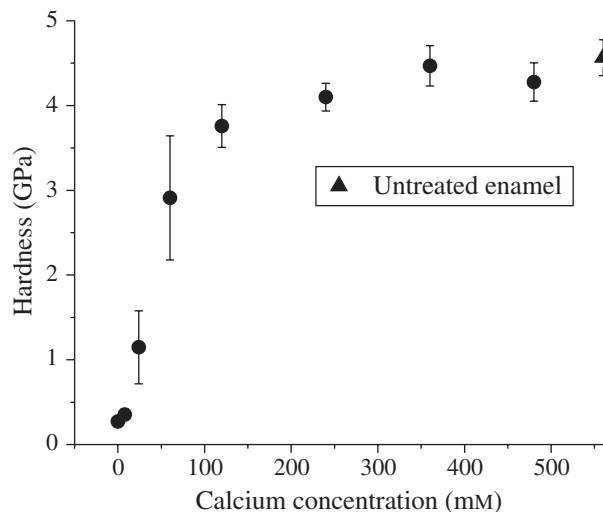

Fig. 2. Hardness of group 2 enamel samples as a function of citric acid solution calcium concentration. Samples were exposed for 300 s.

[Ca] ≥ 120 mM, there was considerably less variation in hardness with calcium concentration. For group 1 (120 s exposure) there was no statistically significant difference between the hardness of samples exposed to solutions with a [Ca] ≥ 120 mM and those of untreated samples. For group 2 (300 s exposure) there was a small but statistically significant difference between the hardness of samples exposed to solutions containing [Ca] = 120 mM and [Ca] = 240 mM, and that of untreated samples, but no such difference for samples exposed to solutions with [Ca] ≥ 360 mM.

The hardness of groups 1 and 2 samples as a function of DS_{HA} is shown in Fig. 3. Although some of the DS_{HA} values in the range are approximate, as indicated, these data demonstrate that virtually no enamel dissolution
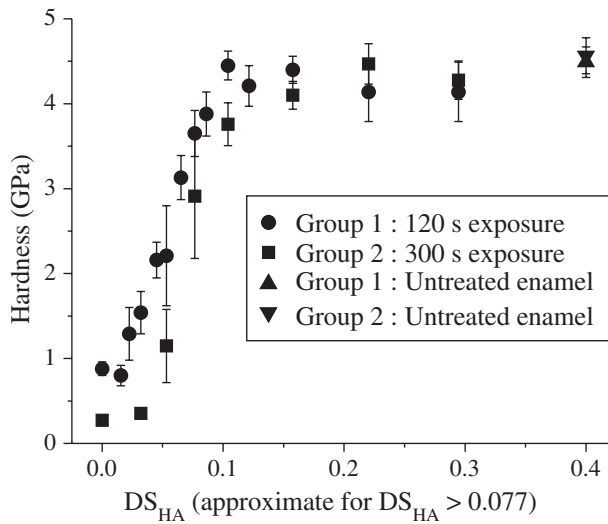
Fig. 3. Hardness of group 1 and 2 enamel samples as a function of citric acid solution DS_{HA}. Group 1 samples are represented by circles and were exposed for 120 s; group 2 are represented by squares and were exposed for 300 s. DS_{HA} values were approximate for $DS_{HA} > 0.077$.

took place in considerably undersaturated solutions. A $[Ca] = 120$ mM corresponded to $DS_{HA} \approx 0.104$. These data are also included to facilitate comparison with other studies in the literature which use DS_{HA} rather than calcium concentration as the factor under investigation, and to explain the use of $DS_{HA} = 0.1$ in sample group 3.

Tapping mode AFM images of an untreated enamel sample and of samples exposed to solutions with $[Ca] = 0$ mM ($DS_{HA} = 0.000$), 16 mM ($DS_{HA} = 0.045$) and 480 mM ($DS_{HA} \approx 0.295$) are shown in Fig. 4A–D, respectively. Shallow scratches arising from the polishing method could be seen on the untreated enamel surface (Fig. 4A). The prismatic structure of enamel could clearly be seen on samples exposed to solutions with $[Ca] = 0$ mM and $[Ca] = 16$ mM, with the prism boundaries raised with respect to the prism cores (Figs 4B,C). In samples exposed to solutions with a $[Ca] = 480$ mM, the prismatic structure of enamel was barely evident, with very slightly raised prism boundaries visible (Fig. 4D).

Group 3 samples

There was a statistically significant difference between the hardness of group 3 samples exposed to different citric acid solutions and that of untreated enamel ($P < 0.00001$). Hardness of the samples as a function of solution is shown in Fig. 5, in which each data point represents a total of 40 hardness measurements on eight enamel samples and error bars represent 95% confidence intervals. Solutions with the same DS_{HA} ($DS_{HA} = 0.101$) but different calcium to phosphate ratios caused a different extent of enamel dissolution. The solution with $[Ca]/[PO_4] = 10$ resulted in a hardness only slightly less
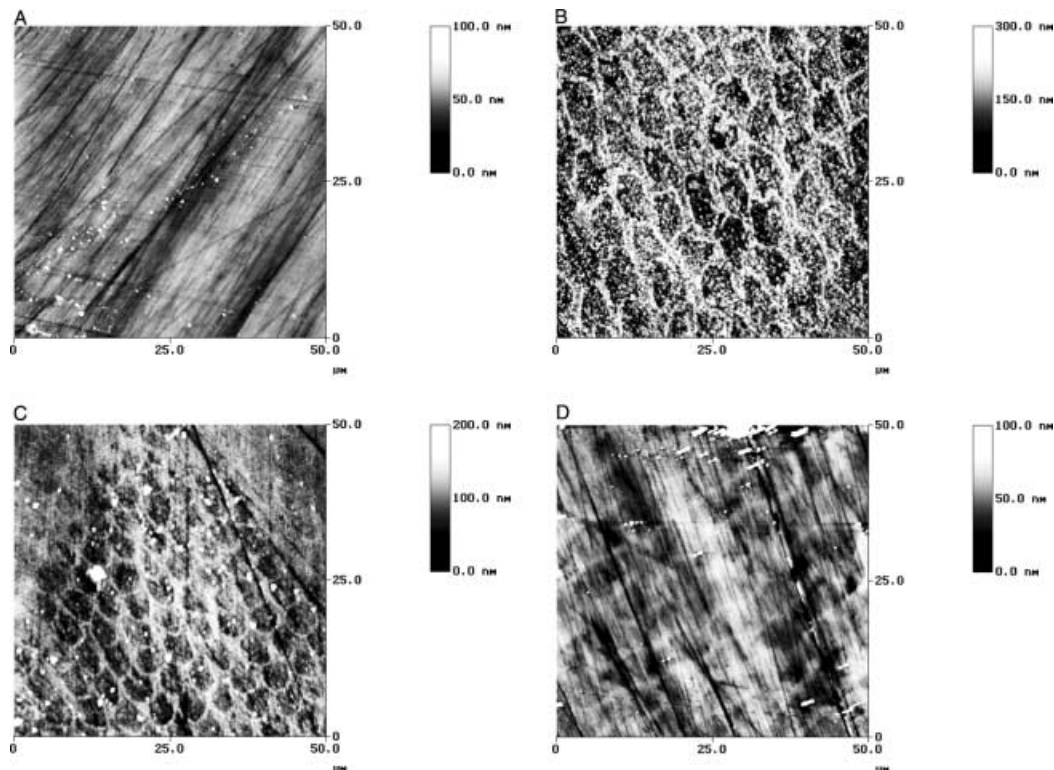

Fig. 4. Tapping mode atomic force microscopy images of an untreated enamel sample (A) and samples after exposure to citric acid solutions with $[Ca] = 0$ mM (B), $[Ca] = 16$ mM (C), and $[Ca] = 480$ mM (D).
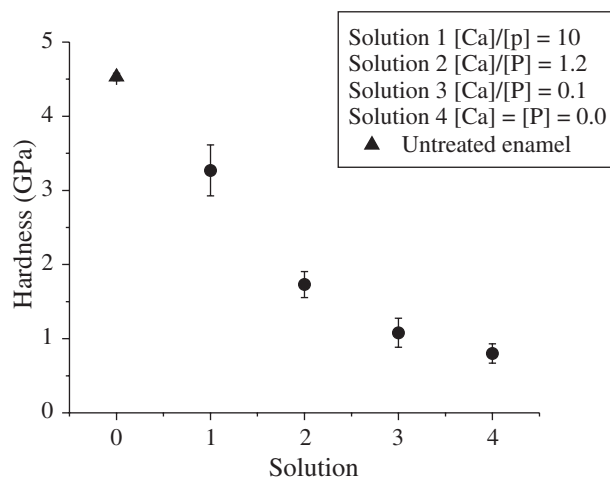
Fig. 5. Hardness of group 3 enamel samples as a function of citric acid solutions with $DS_{HA} = 0.101$ and with different calcium to phosphate ratios as indicated in the legend.

than that of untreated enamel, whereas the solution with $[Ca]/[PO_4] = 0.1$ resulted in a hardness that was not statistically significantly different from that of samples exposed to citric acid without calcium or phosphate. The solution with $[Ca]/[PO_4] = 1.2$ caused an intermediate degree of softening.

Discussion

The aim of this study was to investigate changes in enamel hardness due to dissolution in citric acid solutions with a wide range of calcium concentrations and degrees of saturation with respect to hydroxyapatite, over time-scales typical of clearance times of acids *in vivo* (8). It has been found in previous studies (21 and unpublished observations) that these solutions have constant compositions over the exposure times used in this study. The investigation of the effects of calcium concentration and DS_{HA} may facilitate the formulation of an improved soft drink that has a reduced erosive potential but does not contain more calcium or a higher pH than necessary. This will aid the development of such a drink with a good taste and a suitable shelf life.

Figures 1 and 2 demonstrate that enamel hardness is a complex function of solution calcium concentration, with an approximate threshold condition defined by $[Ca] = 120$ mM, $[PO_4] = 0.57$ mM and pH 3.30. Below this calcium concentration, substantial enamel softening took place, indicating the initial stages of enamel dissolution, while above it there was little or no statistically significant difference between the hardness of treated and untreated samples. Nevertheless, the solutions with a $[Ca] \geq 120$ mM were still considerably undersaturated, as shown in Fig. 3, and the solution with a $[Ca] = 120$ mM had $DS_{HA} \approx 0.104$.

The AFM images of samples exposed to a solution with a $[Ca] = 480$ mM for 120 s (Fig. 4D) showed slight changes in surface structure compared with untreated

enamel (Fig. 4A), in that the prismatic appearance of enamel was just visible. Although there was little or no statistically significant difference, hardness values of those samples exposed to solutions with a $[Ca] > 120$ mM were consistently lower than those of untreated samples. Nevertheless, the AFM results (Fig. 4D) show that some dissolution and/or precipitation must have occurred in solutions with a $[Ca] = 480$ mM. This demonstrates that AFM is very sensitive to the initial stages of enamel dissolution even when the dissolution occurs at very low rates. In contrast, the dissolution of samples treated with solutions with $[Ca] = 0$ mM and $[Ca] = 16$ mM was clearly visible, as shown in Fig. 4B and C, and as indicated by hardness values in Fig. 1. It is probable that a greater change in enamel hardness would be seen for solutions with a $[Ca] > 120$ mM after exposure times longer than 300 s. However, exposure times of 120 s and 300 s were chosen because they are comparable to the clearance time of citric acid in the mouth, and are thought to have a better physiological relevance than longer exposure times (8).

The results of the first part of this study (sample groups 1 and 2) demonstrate that a calcium concentration of 120 mM and a phosphate concentration of 0.57 mM were sufficient to greatly reduce the erosive potential of a citric acid solution at pH 3.30. There was no apparent benefit in using higher concentrations of calcium. Since a solution with a $[Ca] = 120$ mM is considerably undersaturated (DS ≈ 0.104), it is not immediately clear why there was no statistically significant enamel softening above this value, as it cannot be explained in terms of saturation with respect to hydroxyapatite. Related studies over much longer time-scales have shown that no measurable enamel dissolution occurs in acids with $DS_{HA} = 0.4$ and pH 4.3 at less than 96 h exposure (5). Although it has been suggested that the initial phase of enamel dissolution is governed not by the thermodynamics of hydroxyapatite dissolution but by those of other calcium phosphates, the solutions used in this study were also undersaturated with respect to octacalcium phosphate, dicalcium phosphate and tricalcium phosphate, and the lack of dissolution cannot therefore be explained by saturation effects.

There may be kinetic factors responsible for the decrease in enamel dissolution rate in the solutions with high calcium concentrations. It has been shown that calcium ions are detached from the hydroxyapatite surface before phosphate ions (23). The observed effect may be due to the high concentration of calcium ions which inhibit the detachment of calcium ions from the enamel surface.

This study was conducted with an emphasis on enamel erosion caused by soft drinks. The calcium and phosphate concentrations in the first part of this study can be taken as a simple recipe for soft drink formulation in terms of calcium and phosphate concentrations and pH. A reduction in enamel softening of, for example, 80% after 120 s exposure *in vitro* could be achieved by consulting Fig. 1 and selecting a calcium concentration of 60 mM with a phosphate concentration of 0.57 mM and a pH of 3.30. It would be necessary to use an alternative method of pH adjustment, as

the chloride concentration of this solution would clearly be unacceptable in a drink.

The second part of this study was performed to test the assumption that DS_{HA} is the important parameter in determining enamel dissolution rate and that individual calcium and phosphate concentrations are unimportant. The results shown in Fig. 5 clearly demonstrate that this is not the case, since solutions with the same DS_{HA} and different [Ca] and [PO$_4$] resulted in significantly different rates of enamel dissolution. Thus, the assumption employed in a number of models of enamel dissolution (3, 6, 9–12) seems to be invalid, under the experimental conditions used in the present study.

In terms of enamel dissolution models, these results indicate that simple models using DS_{HA} and not including [Ca] and [PO$_4$] are not sufficient to model enamel dissolution. In terms of soft drink modification, these results indicate that DS_{HA} cannot be used to characterize the erosive potential of a drink. In addition, it can be seen that added calcium is substantially more effective than added phosphate at reducing dissolution rate. This should be taken into account when designing soft drinks with a reduced erosive potential.

References

1. PATEL MV, FOX JL, HIGUCHI WI. Effect of acid type on kinetics and mechanism of dental enamel demineralization. *J Dent Res* 1987; **66**: 1425–1430.
2. DOWKER SEP, ANDERSON P, ELLIOT JC, GAO XJ. Crystal chemistry and dissolution of calcium phosphate in dental enamel. *Mineral Mag* 1999; **63**: 791–800.
3. MARGOLIS HC, ZHANG YP, LEE CY, KENT RL jr, MORENO EC. Kinetics of enamel demineralisation *in vitro*. *J Dent Res* 1999; **78**: 1326–1335.
4. SHELLIS RP. A microcomputer program to evaluate the saturation of complex solutions with respect to biominerals. *Comp Appl Biol Sci* 1988; **4**: 373–379.
5. GAO XJ, ELLIOT JC, ANDERSON P. Scanning and contact microradiography study of the effect of degree of saturation on the rate of enamel demineralization. *J Dent Res* 1991; **70**: 1332–1337.
6. ZHANG YP, KENT RL jr, MARGOLIS HC. Enamel demineralization under driving forces found in dental plaque. *Eur J Oral Sci* 2000; **108**: 207–213.
7. TANAKA M, KADOMA Y. Comparative reduction of enamel demineralization by calcium and phosphate in vitro. *Caries Res* 2000; **34**: 241–245.
8. BASHIR E, EKBERG O, LAGERLOF F. Salivary clearance of citric acid after an oral rinse. *J Dent* 1995; **23**: 209–212.
9. MARGOLIS HC, MORENO EC. Kinetics of hydroxyapatite dissolution in acetic, lactic and phosphoric acid solutions. *Calcif Tissue Int* 1992; **50**: 137–143.
10. BUDZ JA, NANCOLLAS GH. The mechanism of dissolution of hydroxyapatite and carbonated apatite in acidic solutions. *J Crystal Growth* 1988; **91**: 490–496.
11. ZHANG J, NANCOLLAS GH. Dissolution deceleration of calcium phosphate crystals at constant undersaturation. *J Crystal Growth* 1992; **123**: 59–68.
12. BUDZ JA, LORE M, NANCOLLAS GH. Hydroxyapatite and carbonated apatite as models for the dissolution behaviour of human dental enamel. *Adv Dent Res* 1987; **1**: 314–321.
13. GRENBY TH. Lessening dental erosive potential by product modification. *Eur J Oral Sci* 1996; **104**: 221–228.
14. ZERO DT. Etiology of dental erosion – extrinsic factors. *Eur J Oral Sci* 1996; **104**: 162–177.
15. FELDMAN M, BARNETT C. Relationships between the acidity and osmolality of popular beverages and reported postprandial heartburn. *Gastroenterology* 1995; **108**: 125–131.
16. LARSEN MJ, NYVAD B. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. *Caries Res* 1999; **33**: 81–87.
17. LARSEN MJ. Degrees of saturation with respect to apatites in fruit juices and acidic drinks. *Scand J Dent Res* 1975; **83**: 13–17.
18. FINKE M, JANDT KD, PARKER DM. The early stages of native enamel dissolution studied with atomic force microscopy. *J Colloid Interface Sci* 2000; **232**: 156–164.
19. FINKE M, HUGHES JA, PARKER DM, JANDT KD. Mechanical properties of *in situ* demineralised human enamel measured by AFM nanoindentation. *Surf Sci* 2001; **491**: 456–467.
20. BARBOUR ME, PARKER DM, JANDT KD. Enamel dissolution as a function of solution degree of saturation with respect to hydroxyapatite. A nanoindentation study. *J Colloid Interface Sci* 2003 (in press).
21. BARBOUR ME, PARKER DM, ALLEN GC, JANDT KD. Human enamel dissolution in citric acid as a function of pH in the range 2.30 < pH < 6.30 – a nanoindentation study. *Eur J Oral Sci* 2003; **111**: 258–262.
22. OLIVER WC, PHARR GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *J Mater Res* 1992; **7**: 1564–1583.
23. DOROZHKIN SV. Surface reactions of apatite dissolution. *J Colloid Interface Sci* 1997; **191**: 489–497.